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(54) Title: METHODS AND COMPOSITIONS TO TREAT CARDIOVASCULAR DISEASE USING 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 OR 93804

(57) Abstract: The present invention relates to methods for the diagnosis and treatment of cardiovascular disease, including, but not limited to, atherosclerosis, reperfusion injury, hypertension, restenosis, arterial inflammation, thrombosis and endothelial cell disorders. Specifically, the present invention identifies the differential expression of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 genes in cardiovascular disease states, relative to their expression in normal, or non-cardiovascular disease states, and/or in response to manipulations relevant to cardiovascular disease. The present invention de-scribes methods for the diagnostic evaluation and prognosis of various cardiovascular diseases, and for the identification of subjects exhibiting a predisposition to such conditions. The invention also provides methods for identifying a compound capable of modu-lating cardiovascular disease. The present invention also provides methods for the identification and therapeutic use of compounds as treatments of cardiovascular disease.

METHODS AND COMPOSITIONS TO TREAT CARDIOVASCULAR DISEASE USING 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 OR 93804

5   This application claims priority to U.S. provisional application number 60/339,582, filed November 5, 2001, the entire contents of which are incorporated herein by reference.

Cardiovascular disease is a major health risk throughout the industrialized world. Atherosclerosis, the most prevalent of cardiovascular diseases, is the principal cause of heart attack, stroke, and peripheral vascular disease resulting in significant disability and

10   limb loss, and thereby the principle cause of death in the United States.

Atherosclerosis is a complex disease involving aspects of lipid metabolism and vascular inflammation. Both have significant effects on the initiation and progression of atherosclerosis. Irregular lipid metabolism is a very well established risk factor for atherosclerosis. Elevated low density lipoprotein (LDL), very low density lipoproteins (VLDL), triglycerides and low levels of

15   high density lipoproteins (HDL) all independently contribute to atherosclerosis development and/or progression. There are a number of effective therapies currently being utilized in the clinic that result in lowering of these risk factors and, in turn decrease the rate of mortality and morbidity associated with atherosclerotic disease. Some of these therapies include the cholesterol lowering drugs statins, the triglyceride lowering drugs fibrates and niacin and the triglyceride lowering/HDL

20   raising PPAR alpha activators. There is a need to identify new targets for atherosclerosis therapy.

There have been significant advances made in understanding the role that inflammation plays in the process of atherosclerosis. Atherosclerosis involves many cell types and molecular factors (described in, for example, Ross (1993) *Nature* 362: 801-809). The process, in normal circumstances a protective response to insults to the endothelium

25   and smooth muscle cells (SMCs) of the wall of the artery, consists of the formation of fibrofatty and fibrous lesions or plaques, preceded and accompanied by inflammation. The advanced lesions of atherosclerosis may occlude the artery concerned, and result from an excessive inflammatory-fibroproliferative response to numerous different forms of insult. Injury or dysfunction of the vascular endothelium is a common feature of many conditions

30   that predispose an individual to accelerated development of atherosclerotic cardiovascular disease. There has been considerable effort in establishing that hypertension contributes to atherosclerosis. The identification of molecules that regulate blood pressure and vascular tone will be useful in discovering new therapies to treat cardiovascular diseases such as atherosclerosis.

The present invention provides methods and compositions for the diagnosis and

35   treatment of cardiovascular disease. As used herein, disorders involving the heart, or

"cardiovascular disease" or a "cardiovascular disorder" include a disease or disorder which affects the cardiovascular system, e.g., the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an imbalance in arterial pressure, a malfunction of the heart, or an occlusion of a blood vessel, e.g., by a thrombus. A cardiovascular

5   disorder includes, but is not limited to disorders such as arteriosclerosis, atherosclerosis, cardiac hypertrophy, ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, valvular disease, including but not limited to,

10   valvular degeneration caused by calcification, rheumatic heart disease, endocarditis, or complications of artificial valves; atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, pericardial disease, including but not limited to, pericardial effusion and pericarditis; cardiomyopathies, e.g., dilated cardiomyopathy or idiopathic cardiomyopathy,

15   myocardial infarction, coronary artery disease, coronary artery spasm, ischemic disease, arrhythmia, sudden cardiac death, and cardiovascular developmental disorders (e.g., arteriovenous malformations, arteriovenous fistulae, raynaud's syndrome, neurogenic thoracic outlet syndrome, causalgia/reflex sympathetic dystrophy, hemangioma, aneurysm, cavernous angioma, aortic valve stenosis, atrial septal defects, atrioventricular canal,

20   coarctation of the aorta, ebsteins anomaly, hypoplastic left heart syndrome, interruption of the aortic arch, mitral valve prolapse, ductus arteriosus, patent foramen ovale, partial anomalous pulmonary venous return, pulmonary atresia with ventricular septal defect, pulmonary atresia without ventricular septal defect, persistance of the fetal circulation, pulmonary valve stenosis, single ventricle, total anomalous pulmonary venous return,

25   transposition of the great vessels, tricuspid atresia, truncus arteriosus, ventricular septal defects). A cardiovascular disease or disorder also can include an endothelial cell disorder.

As used herein, an "endothelial cell disorder" includes a disorder characterized by aberrant, unregulated, or unwanted endothelial cell activity, e.g., proliferation, migration, angiogenesis, or vascularization; or aberrant expression of cell surface adhesion molecules

30   or genes associated with angiogenesis, e.g., TIE-2, FLT and FLK. Endothelial cell disorders include tumorigenesis, tumor metastasis, psoriasis, diabetic retinopathy, endometriosis, Grave's disease, ischemic disease (e.g., atherosclerosis), and chronic inflammatory diseases (e.g., rheumatoid arthritis).

A cardiovascular disease can also include thrombosis. Thrombosis can result from platelet dysfunction, e.g. seen in myocardial infarction, angina, hypertension, lipid disorders, diabetes mellitus; myelodysplastic syndromes; myeloproliferative yndromes (including polycythemia vera and thombocythemia); thrombotic thrombocytopenic purpuras; HIV-induced platelet disorders (AIDS-Thrombocytopenia); heparin induced thrombocytopenia; mural cell alterations/interactions leading to platelet aggregation/degranulation, vascular endothelial cell activation/injury, monocyte/macrophage extravasation and smooth muscle cell proliferation; autoimmune disorders such as, but not limited to vasculitis, antiphospholipid syndromes, systemic lupus erythromatosis; inflammatory diseases, such as, but not limited to iImmune activation; graft Vs host disease; radiation induced hypercoagulation; clotting factor dysregulation either hereditary (autosomal dominant or recessive) such as, but not limited to clotting factor pathways including protein C/S, Anti-thrombin III deficiency, and the Factor V Leiden mutation or acquired such as but not limited to autoimmune, cancer -associated and drug-induced dysregulation of clotting factors.

"Treatment", as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease or disorder, a symptom of disease or disorder or a predisposition toward a disease or disorder, with the purpose of curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving or affecting the disease or disorder, at least one symptom of disease or disorder or the predisposition toward a disease or disorder. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides. Representative molecules are described herein.

The present invention is based, at least in part, on the discovery that nucleic acid and protein molecules, (described infra), are differentially expressed in cardiovascular disease states relative to their expression in normal, or non-cardiovascular disease states. The modulators of the molecules of the present invention, identified according to the methods of the invention can be used to modulate (e.g., inhibit, treat, or prevent) or diagnose cardiovascular disease, including, but not limited to, atherosclerosis and thrombosis.

"Differential expression", as used herein, includes both quantitative as well as qualitative differences in the temporal and/or tissue expression pattern of a gene. Thus, a differentially expressed gene may have its expression activated or inactivated in normal

versus cardiovascular disease conditions (for example, in an experimental cardiovascular disease system such as in an animal model for atherosclerosis). The degree to which expression differs in normal versus cardiovascular disease or control versus experimental states need only be large enough to be visualized via standard characterization techniques,

5 e.g., quantitative PCR, Northern analysis, subtractive hybridization. The expression pattern of a differentially expressed gene may be used as part of a prognostic or diagnostic cardiovascular disease, e.g., artherosclerosis and/or thrombosis, evaluation, or may be used in methods for identifying compounds useful for the treatment of cardiovascular disease, e.g., atherosclerosis and/or thrombosis. In addition, a differentially expressed gene

10 involved in cardiovascular disease may represent a target gene such that modulation of the level of target gene expression or of target gene product activity will act to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect a cardiovascular disease condition, e.g., atherosclerosis and/or thrombosis. Compounds that modulate target gene expression or activity of the target gene product can be used in the treatment of

15 cardiovascular disease. Although the genes described herein may be differentially expressed with respect to cardiovascular disease, and/or their products may interact with gene products important to cardiovascular disease, the genes may also be involved in mechanisms important to additional cardiovascular cell processes.

20 **Gene ID 139**

The human 139 sequence (SEQ ID NO:1), (GI: 181948, known also as endothelial differentiation protein (EDG-1)) which is approximately 2757 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1143 nucleotides, including the termination codon. The coding sequence encodes a 381

25 amino acid protein (SEQ ID NO:2) (GI: 181949).

As determined by TaqMan analysis, expression of 139 mRNA was seen in human liver, skin, tonsil, spleen, bone marrow and peripheral blood cells. When marmosets are treated with the statin cerivastatin, expression of 139 mRNA was reduced compared to a placebo. Statins are used clinically for the reduction of cholesterol, a defined risk factor

30 for atherosclerosis/coronary artery disease. Genes that are regulated by statins in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of this agent and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. Therefore, 139 is predicted to be

responsible for the therapeutic benefit of cerivastatin in lower cholesterol and triglyceride levels and is useful in the treatment of cardiovascular disease.

Gene ID 258

5   The human 258 sequence (SEQ ID NO:3), (GI: 466488), known also as arginine vasopressin receptor (AVPR1)) which is approximately 1492 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1254 nucleotides, including the termination codon. The coding sequence encodes a 418 amino acid protein (SEQ ID NO:4) (GI:667068).

10   A determined by TaqMan analysis, expression of 258 mRNA was most abundant in normal human liver with moderate expression in breast and skin decubitus, with lower amounts seen in normal skin, skeletal muscle, adipose tissue and heart. 258 mRNA was repressed in the livers of monkeys fed a high cholesterol diet. Cholesterol is a well-established risk factor for the development and progression of atherosclerosis /coronary

15   artery disease. Genes that are regulated by cholesterol in animal models and/or in vitro models are predicted to be responsible for disease progression and or initiation and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. Therefore, antagonists of 258 are predicted to lower cholesterol and triglyceride levels.

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Gene ID 1261

The human 1261 sequence (SEQ ID NO:5), (GI:1813881, known also as NAK-1) which is approximately 2481 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1794 nucleotides, including the

25   termination codon. The coding sequence encodes a 598 amino acid protein (SEQ ID NO:6) (GI:1813882).

As determined by TaqMan analysis, expression of human 1261 mRNA was upregulated in human microvascular endothelial cells (HMVEC) from the heart when they were treated with the pro-angiogenic factors vascular endothelial growth factor (VEGF),

30   tumor necrosis factor alpha (TNF-alpha) and interleukin 1 beta (IL-1beta). 1261 mRNA was also upregulated when HMVEC cells were treated with serum. These data indicate that 1261 mRNA is upregulated when exposed to pro-angiogenic factors which indicates that it plays a role in angiogenesis. When human umbilical vein endothelial cells were

subjected to laminar flow sheer stress (a condition known to promote the formation of atherosclerosis) 1261 mRNA was increased compared to cells not exposed to sheer stress.

Gene ID 1486

5   The human 1486 sequence (SEQ ID NO:7), (GI:791187, known also as JNK activating kinase (JNKK1)) which is approximately 1310 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1197 nucleotides, including the termination codon. The coding sequence encodes a 399 amino acid protein (SEQ ID NO:8) (GI:791188)..

10   As determined by taqMan analysis, expression of 1486 mRNA was found to be high in human liver as compared to other normal tissues tested. Furthermore, 1486 mRNA was repressed in livers of marmosets when they were treated with probucol or a combination of cerivastatin and fenofibrate. Probucol is used clinically for the treatment of atherosclerosis /coronary artery disease. Genes that are regulated by probucol in animal

15   models and/or in vitro models are predicted to be responsible for the therapeutic benefit of this agent and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders.

Fibrates are used clinically for the reduction of triglycerides and statins for the reduction of cholesterol, both well-defined risk factor for atherosclerosis/coronary artery

20   disease. Genes that are regulated by this combination treatment in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of these agents and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders.

Repression of 1486 is predicted to lower cholesterol and triglyceride levels and is

25   thus useful in treating cardiovascular disease.

Gene ID 2398

The human 2398 sequence (SEQ ID NO:9), (GI:7363341, known also as C-C chemokine receptor type 11 (CC CKR-11)) which is approximately 1113 nucleotides long

30   including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1050 nucleotides, including the termination codon. The coding sequence encodes a 350 amino acid protein (SEQ ID NO:10) (GI:7363342).

As determined by TaqMan analysis, expression of 2398 mRNA is down-regulated in endothelial cells (ECs) by shear stress, correlating with its anti-atherogenic or anti-

proliferative effects. The laminar shear stress (LSS) paradigm is designed to simulate blood flow in a straight unbranched stretch of artery (treatment is 10 dyn/cm² LSS for 24h). In vitro, it is known to increase nitric oxide production, decrease adhesion molecule expression and proliferation, and protect cells from the normal apoptotic response to serum

5   withdrawal. Because of these effects in vitro, the paradigm is thought to reproduce the athero-protected (relatively lesion-free) state that is observed in straight, unbranched arteries experiencing laminar shear stress in vivo.

2398 is upregulated in ECs by hypoxic conditions at 24h. VEGF is upregulated at 6h. Given that 2398 is also upregulated by VEGF, its induction by hypoxia may be

10   downstream of VEGF. Modulators of 2398 expression would be useful in treating cardiovascular disorders including but not limited to atherosclerosis.

Gene ID 2414

The human 2414 sequence (SEQ ID NO:11), (GI:1457938, known also as GPR68))

15   which is approximately 1697 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1095 nucleotides, including the termination codon. The coding sequence encodes a 365 amino acid protein (SEQ ID NO:12) GI:1457939).

As assessed by TaqMan analysis, expression of 2414 mRNA is up-regulated during

20   the monocyte/macrophage lipid-loading process. Monocyte/macrophages that are stimulated with modified LDL mimics foam cell formation. Foam cells are the initiating event of atherogenesis, thus the genes that are up-regulated in this process have utility in identifying compounds useful in treating atherogenesis.

As assessed by TaqMan analysis, expression of 2414 mRNA is up-regulated during

25   various cytokine stimulations in endothelial cells and monoctye/macrophages. CD40L, TNFalpha, IL-1, and IFNgamma were used to stimulate either endothelial cells or monocyte/macrophages. These cytokines initiate/sustain the atherosclerotic pathogenesis.

Expression of 2414 mRNA is up-regulated in monkey atheroma samples compared to normal vessels. This was observed both quantitatively by TaqMan analysis and

30   qualitatively by in situ hybridization. Six different monkey atheroma samples were compared with normal vessel for quantitative comparison of 2414 expression. Consistently, there was increased expression of 2414 mRNA in atheromas as compared to normal vessels.

2414 shows up-regulation during many in vivo and in vitro paradigms that mimic atherogenesis. Therefore, 2414 blockade will lower the lesion formation in atherosclerotic vessels thereby attenuating the progression of atherogenesis.

Gene ID 7660

The human 7660 sequence (SEQ ID NO:13), (GI:2213808), known also as JA61)) which is approximately 1181 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1152 nucleotides, including the termination codon. The coding sequence encodes a 384 amino acid protein (SEQ ID NO:14) GI:2213809).

As assessed by TaqMan analysis, expression of 7660 mRNA is restricted to normal human liver. In addition, 7660 mRNA was reduced in marmoset liver which had been treated with cerivastatin. Statins are used clinically for the reduction of cholesterol, a defined risk factor for atherosclerosis/coronary artery disease. Genes that are regulated by statins in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of this agent and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders.

Regulation of 7660 is predicted to reduce total cholesterol and have beneficial effects against atherosclerosis and hyperlipidemias.

Gene ID 8587

The human 8587 sequence (SEQ ID NO:15), (GI:410213, known also as sodium/bile acid cotransporter) which is approximately 1580 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1047 nucleotides, including the termination codon. The coding sequence encodes a 349 amino acid protein (SEQ ID NO:16) (GI:410214).

As assessed by TaqMan analysis, expression of 8587 mRNA is high in human liver. In addition 8587 mRNA is upregulated by cholestyramine in vivo in marmoset liver model and by combination statin/fibrate therapy in vivo in marmoset liver model. Cholestyramine is used clinically for the reduction of cholesterol, a defined risk factor for atherosclerosis/coronary artery disease. Genes that are regulated by cholestyramine in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of this agent and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. Fibrates are used

clinically for the reduction of triglycerides and statins for the reduction of cholesterol, both well-defined risk factors for atherosclerosis/coronary artery disease. Genes that are regulated by this combination treatment in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of these agents and represent new

5   therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders.

Repression of 8587 is predicted to reduce total cholesterol and triglycerides and therefore has a role in cholesterol metabolism.

10   **Gene ID 10183**

The human 10183 sequence (SEQ ID NO:17), (GI:189328, known also as ornithine aminotransferase) which is approximately 2013 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1317 nucleotides, including the termination codon. The coding sequence encodes a 439 amino

15   acid protein (SEQ ID NO:18) (GI:189329).

As assessed by TaqMan analysis, 10183 mRNA is repressed by probucol and cerivastatin/fenofibrate in in vivo marmoset models. Probucol is used clinically for the treatment of atherosclerosis /coronary artery disease. Genes that are regulated by probucol in animal models and/or in vitro models are predicted to be responsible for the therapeutic

20   benefit of this agent and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. Fibrates are used clinically for the reduction of triglycerides and statins for the reduction of cholesterol, both well-defined risk factor for atherosclerosis/coronary artery disease. Genes that are regulated by this combination treatment in animal models and/or in vitro models are

25   predicted to be responsible for the therapeutic benefit of these agents and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. 10183 mRNA is also down-regulated by statin and PPAR agonists in vitro in primary human hepatocytes. PPAR alpha agonists are used clinically for the reduction of triglycerides and statins for the reduction of cholesterol, both well-

30   defined risk factor for atherosclerosis/coronary artery disease. Genes that are regulated by this combination treatment in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of these agents and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. High expression of 10183 mRNA was seen in human liver.

Repression of 10183 polynucleotides or polypeptides is predicted to reduce total cholesterol and triglyceride levels and thus is useful in treating cardiovascular disease.

Gene ID 10550

5   The human 10550 sequence (SEQ ID NO:19), (GI:9409793, known also as succinyl-CoA-synthetase) which is approximately 1227 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 999 nucleotides, including the termination codon. The coding sequence encodes a 333 amino acid protein (SEQ ID NO:20) (GI:9409794).

10   As assessed by TaqMan analysis, 10550 mRNA is repressed by probucol and cerivastatin/fenofibrate in in vivo marmoset models. Probucol is used clinically for the treatment of atherosclerosis /coronary artery disease. Genes that are regulated by probucol in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of this agent and represent new therapeutic targets for the treatment of

15 cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. Fibrates are used clinically for the reduction of triglycerides and statins for the reduction of cholesterol, both well-defined risk factor for atherosclerosis/coronary artery disease. Genes that are regulated by this combination treatment in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of these agents and represent new

20 therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. High expression was also seen in human liver.

      Repression of 10550 synthetase predicted to reduce total cholesterol and triglycerides.

Gene ID 12680

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      The human 12680 sequence (SEQ ID NO:21), (GI:3006201, known also as prostaglandin transporter (PT)) which is approximately 4040 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1929 nucleotides, including the termination codon. The coding sequence encodes a 643

30 amino acid protein (SEQ ID NO:22)(GI:1617590).

      As assessed by TaqMan analysis, 12680 mRNA is repressed by probucol and cerivastatin/fenofibrate in in vivo marmoset models. Probucol is used clinically for the treatment of atherosclerosis /coronary artery disease. Genes that are regulated by probucol in animal models and/or in vitro models are predicted to be responsible for the therapeutic

benefit of this agent and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. Fibrates are used clinically for the reduction of triglycerides and statins for the reduction of cholesterol, both well-defined risk factor for atherosclerosis/coronary artery disease. Genes that are

5   regulated by this combination treatment in animal models and/or in vitro models are predicted to be responsible for the therapeutic benefit of these agents and represent new therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. High expression was also seen in human liver.

Repression of 12680 synthetase predicted to reduce total cholesterol and

10   triglycerides.

Gene ID 17921

The human 17921 sequence (SEQ ID NO:23) which is approximately 4897 nucleotides long including untranslated regions, contains a predicted methionine-initiated

15   coding sequence of about 1518 nucleotides, including the termination codon. The coding sequence encodes a 506 amino acid protein (SEQ ID NO:24). (Partial sequence of 4312 nucleotides, known also as KIAA1382 is disclosed as GI:7243144).

The rat ortholog of 17921 has been functionally characterized and shown to increase net levels of amino acids inside cells. It transports alanine with highest affinity,

20   and also transports glutamine. Intracellular glutamine can inhibit the activity of endothelial nitric oxide synthase (eNOS).

Amino acid transporter 17921 is most highly expressed in endothelial cells, and is also highly expressed in vascular smooth muscle. In endothelial cells, expression of 17921 is strongly and consistently upregulated by laminar shear stress. 17921 is highly

25   expressed in all human vessels examined .

Given the high expression level of 17921 in endothelial cells, the net transfer of glutamine may be quite significant, and may result in reduced nitric oxide production. This would have consequences relevant to angiogenesis, atherosclerosis, and vascular tone. Nitric oxide is a major vasodilator. Mice lacking eNOS have elevated blood pressure,

30   increased succeptibility to atherosclerosis, and insufficient angiogenesis. Amino acid transporter 17921 may modulate the ability of endothelial cells to undergo angiogenesis by the additional mechanism of contributing to the amino acid pool available for protein synthesis.

Gene ID 32248

The human 32248 sequence (SEQ ID NO:25), (GI:8439650, known also as acetyl –
CoA synthetase) which is approximately 2106 nucleotides long including untranslated
regions, contains a predicted methionine-initiated coding sequence of about 2103

5   nucleotides, including the termination codon. The coding sequence encodes a 701 amino
acid protein (SEQ ID NO:26) (GI:8439651).

As assessed by TaqMan analysis, 32248 mRNA was repressed by n-3
polyunsaturated fatty acid in monkey liver model. Dietary polyunsaturated fatty acid has
been shown to protect against the development of atherosclerosis /coronary artery disease.

10   Genes that are regulated by polyunsaturated fatty acids in animal models and/or in vitro
models are predicted to be responsible for the therapeutic benefit of this agent and
represent new therapeutic targets for the treatment of cardiovascular
disease/atherosclerosis/ dyslipidemia/lipid disorders. 32248 mRNA was up-regulated in
liver by cholestyramine and the combination statin/fibrate therapy in marmoset liver

15   model. Cholestyramine is used clinically for the reduction of cholesterol, a defined risk
factor for atherosclerosis/coronary artery disease. Genes that are regulated by
cholestyramine in animal models and/or in vitro models are predicted to be responsible for
the therapeutic benefit of this agent and represent new therapeutic targets for the treatment
of cardiovascular disease/atherosclerosis/ dyslipidemia/lipid disorders. Fibrates are used

20   clinically for the reduction of triglycerides and statins for the reduction of cholesterol, both
well-defined risk factor for atherosclerosis/coronary artery disease. Genes that are
regulated by this combination treatment in animal models and/or in vitro models are
predicted to be responsible for the therapeutic benefit of these agents and represent new
therapeutic targets for the treatment of cardiovascular disease/atherosclerosis/

25   dyslipidemia/lipid disorders. 32248 was highly expressed in human liver.

Regulation of 32248 predicted to reduce total cholesterol and triglycerides.

Gene ID 60489

The human 60489 sequence (SEQ ID NO:27), which is approximately 1255

30   nucleotides long including untranslated regions, contains a predicted methionine-initiated
coding sequence of about 1023 nucleotides, including the termination codon. The coding
sequence encodes a 341 amino acid protein (SEQ ID NO:28).

As assessed by TaqMan analysis, 60489 mRNA expression is restricted to the
liver, colon and small intestine in the human. In addition, 60489 mRNA was upregulated

in human hepatocytes that were treated with statins or PPAR alpha, either alone or in combination. 60489 is a novel member of the diacylglycerol acyltransferase family. These molecules are known to play a key role in triglyceride biosynthesis. Targeted inhibition of 60489 would be predicted to result in decreased triglycerides and thus be protective against atherosclerosis and hyperlipidemia.

Gene ID 93804

The human 93804 sequence (SEQ ID NO:29), (GI:4154286, known also as napsin A) which is approximately 1365 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1260 nucleotides, including the termination codon. The coding sequence encodes a 420 amino acid protein (SEQ ID NO:30) (GI:4154287).

93804, is regulated in in vitro and in vivo models of atherosclerosis as assessed by TaqMan analysis and in situ hybridization.93804 mRNA is up-regulated during lipid-loading in monocytes and macrophages. One of the hallmarks of the beginning of atherogenesis is the formation of lipid-laden macroophages. 93804, shows transcriptional up-regulation during a lipid-loading paradigm in an in vitro monocyte/macrophage culture system. Multiple studies with multiple donors were performed. In one of the donor studies (donor one), the monocytes were stimulated with moderately oxdized LDL (ox-LDL) for various times; 6hours, 48 hours and 72 hours. The stimulation by the modified ox-LDL loads the lipids in monocytes. 93804 exhibited minor up-regulation, 1.3X, during 6 hours stimulation via moderately oxdized LDL. In a long-term stimulation by modified ox-LDL, 93804 showed moderate up-regulation, 7.2X, during the lipid-loading process in monoctyes.

In one of the other donor studies (donor six), the monocytes were stimulated with various modified LDLs; mildly oxdized LDL, moderately oxdized LDL, and acetylated LDL. 93804 was upregulated by all three reagents.

93804 was up-regulated in lesion the prone region of ApoE deficient vessels. ApoE deficient animals form atherosclerotic lesions spontaneously. The lesions are formed in the aortic root area and an arch portion of the asending aorta while very little to no lesions are formed in the desending aorta. 93804 showed greater expression in ApoE deficient animals in athero-lesion enriched region compared to non-lesion area of aorta. This was observed quantitatively by TaqMan experiments. In particular, the expression of

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93804 was higher in the arch region of the aorta when it was compared to the region of the desending aorta.

93804 was up-regulated in a monkey atheroma model. 93804 showed elevated expression in the monkey atheroma vessels compared to nomal vessels by TaqMan

5 experiments. Among three normal monky vessels observed for this study, the expression of 93804 mRNA was not detected. Among six monkey atheroma vessels observed for this study, the expression of 93804 mRNA was detected in four atheroma samples.

93804 shows very restricted expression among various human organs. The highest expression was found in lung. Low to midium expression was shown in tonsil, lymph

10 node, brain and kidney.

Atherosclerosis is considered a chronic inflammatory disease, resulting from interaction between activated monocyte/macropohages, endothelial cells, smooth muscle cells, and humoral and cell-mediated immunological response of arterial wall. For this reason, the identification of genes that are involved in these vascular mechanisms can lead

15 to novel and effective therapeutic targets to prevent atherosclerosis.

93804 belongs to a family of aspartic proteases. Aspartic proteinases are classified by having two aspartic residues that are responsible for the catalytic mechanism. Several well-defined enzymes belong to this family of enzyme: Pepsin, gastricsin, renin, catehpsins, and HIV protease. During last three decades, the family of aspartic proteinases

20 were studied intensely due to their important roles in physiology/pathophysiology.

93804 shows up-regulation during lipid-loading process in monocytemacrophage suggesting its role in atherogenesis. 93804 blockade might lower the lesion formation in atherosclerotic vessels, thereby attenuating the progression of atherogenesis. 93804, is regulated in both in vitro and in vivo paradigms that represent the atherogenesis processes.

25 93804 is up-regulated during lipid-loading process in monocye/macropahge in an in vitro study. In addition, 93804 showed increased expression in ApoE deficient animals in athero-lesion enriched region compared to non-lesion area of aorta. These regulations strongly suggest the atherogenic effect of 93804. Therefore, inhibition of 93804 would result in a decrease in atherogenesis.

30 Various aspects of the invention are described in further detail in the following subsections:

I.   Screening Assays:

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules (organic or inorganic) or other drugs) which bind to

5   139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins, have a stimulatory or inhibitory effect on, for example, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity, or have a stimulatory or inhibitory effect

10   on, for example, the expression or activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate. Compounds identified using the assays described herein may be useful for treating cardiovascular diseases, *e.g.*, atherosclerosis and/or thrombosis.

These assays are designed to identify compounds that bind to a 139, 258, 1261,

15   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, bind to other intracellular or extracellular proteins that interact with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, and interfere with the interaction of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein with other

20   intercellular or extracellular proteins. For example, in the case of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, which is a transmembrane receptor-type protein, such techniques can identify ligands for such a receptor. A 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein ligand or substrate can, for example,

25   be used to ameliorate cardiovascular diseases, *e.g.*, atherosclerosis, ischemia/reperfusion, hypertension, restenosis, arterial inflammation, thrombosis and endothelial cell disorders. Such compounds may include, but are not limited to peptides, antibodies, or small organic or inorganic compounds. Such compounds may also include other cellular proteins.

Compounds identified via assays such as those described herein may be useful, for

30   example, for ameliorating cardiovascular disease, *e.g.*, athersclerosis and/or thrombosis. In instances whereby a cardiovascular disease condition results from an overall lower level of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression and/or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein in a cell or tissue,

compounds that interact with the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein may include compounds which accentuate or amplify the activity of the bound 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. Such compounds would bring about an effective increase in the level of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein activity, thus ameliorating symptoms.

In other instances, mutations within the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene may cause aberrant types or excessive amounts of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins to be made which have a deleterious effect that leads to a cardiovascular disease. Similarly, physiological conditions may cause an excessive increase in 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression leading to a cardiovascular disease. In such cases, compounds that bind to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein may be identified that inhibit the activity of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. Assays for testing the effectiveness of compounds identified by techniques such as those described in this section are discussed herein.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or polypeptide or biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide,

non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.*
5   (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992)
10   *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-
15   310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate 139, 258, 1261, 1486, 2398,
20   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity is determined. Determining the ability of the test compound to modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity can be accomplished by monitoring, for example, intracellular calcium, IP_3, cAMP, or diacylglycerol concentration, the phosphorylation profile of intracellular
25   proteins, cell proliferation and/or migration, gene expression of, for example, cell surface adhesion molecules or genes associated with angiogenesis, or the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -regulated transcription factor. The cell can be of mammalian origin, *e.g.*, an endothelial cell. In one embodiment, compounds that interact with a receptor domain can
30   be screened for their ability to function as ligands, *i.e.*, to bind to the receptor and modulate a signal transduction pathway. Identification of ligands, and measuring the activity of the ligand-receptor complex, leads to the identification of modulators (*e.g.*, antagonists) of this interaction. Such modulators may be useful in the treatment of cardiovascular disease.

The ability of the test compound to modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 binding to a substrate or to bind to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can also be determined. Determining the ability of the test

5   compound to modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 binding to a substrate can be accomplished, for example, by coupling the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate with a radioisotope or enzymatic label such that binding of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

10   12680, 17921, 32248, 60489 or 93804 substrate to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be determined by detecting the labeled 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate in a complex. 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 could also be coupled

15   with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 binding to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate in a complex. Determining the ability of the test compound to bind 139, 258, 1261, 1486, 2398, 2414,

20   7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be accomplished, for example, by coupling the compound with a radioisotope or enzymatic label such that binding of the compound to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be determined by detecting the labeled 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

25   93804 compound in a complex. For example, compounds (e.g., 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 ligands or substrates) can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Compounds can further be enzymatically labeled with, for example, horseradish

30   peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of this invention to determine the ability of a compound (e.g., a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

32248, 60489 or 93804 ligand or substrate) to interact with 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 without the labeling of any of the interactants. For example, a microphysiometer can be used to detect the interaction of a compound with 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

5   10550, 12680, 17921, 32248, 60489 or 93804 without the labeling of either the compound or the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 (McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable

10   potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 .

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

15   32248, 60489 or 93804 target molecule (*e.g.*, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate) with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule. Determining the ability of the test

20   compound to modulate the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule can be accomplished, for example, by determining the ability of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to bind to or interact with the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

25   17921, 32248, 60489 or 93804 target molecule.

Determining the ability of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or a biologically active fragment thereof, to bind to or interact with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule can be

30   accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to bind to or interact with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule can be accomplished by determining the

activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.*, intracellular Ca^{2+}, diacylglycerol, IP_3, cAMP), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (comprising a

5   target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a target-regulated cellular response (*e.g.*, gene expression).

In yet another embodiment, an assay of the present invention is a cell-free assay in which a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

10   32248, 60489 or 93804 protein or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to bind to the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or biologically active portion thereof is determined. Preferred biologically active portions of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

15   60489 or 93804 proteins to be used in assays of the present invention include fragments which participate in interactions with non-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 molecules, *e.g.*, fragments with high surface probability scores. Binding of the test compound to the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein

20   can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or biologically active portion thereof with a known compound which binds 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 to form an assay

25   mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, wherein determining the ability of the test compound to interact with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein comprises

30   determining the ability of the test compound to preferentially bind to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 or biologically active portion thereof as compared to the known compound. Compounds that modulate the interaction of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 with a known target protein may be useful in

regulating the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, especially a mutant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein.

In another embodiment, the assay is a cell-free assay in which a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or biologically active portion thereof is determined. Determining the ability of the test compound to modulate the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein can be accomplished, for example, by determining the ability of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to bind to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule by one of the methods described above for determining direct binding. Determining the ability of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to bind to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule can also be accomplished using a technology such as real-time Biomolecular Interaction Analysis (BIA) (Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore). Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In another embodiment, determining the ability of the test compound to modulate the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein can be accomplished by determining the ability of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to further modulate the activity of a downstream effector of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined or the binding of the effector to an appropriate target can be determined as previously described.

In yet another embodiment, the cell-free assay involves contacting a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or biologically active portion thereof with a known compound which binds the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

5   60489 or 93804 protein to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, wherein determining the ability of the test compound to interact with the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

10   60489 or 93804 protein comprises determining the ability of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to preferentially bind to or modulate the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 target molecule.

In more than one embodiment of the above assay methods of the present invention,

15   it may be desirable to immobilize either 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

20   93804 protein, or interaction of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which

25   adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then

30   combined with the test compound or the test compound and either the non-adsorbed target protein or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound

components, the matrix immobilized in the case of beads, complex determined either
directly or indirectly, for example, as described above. Alternatively, the complexes can
be dissociated from the matrix, and the level of 139, 258, 1261, 1486, 2398, 2414, 7660,
8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 binding or activity determined

5   using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the
screening assays of the invention. For example, either a 139, 258, 1261, 1486, 2398, 2414,
7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or a 139, 258,
1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

10   93804 target molecule can be immobilized utilizing conjugation of biotin and streptavidin.
Biotinylated 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,
32248, 60489 or 93804 protein or target molecules can be prepared from biotin-NHS (N-
hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce
Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well

15   plates (Pierce Chemical). Alternatively, antibodies reactive with 139, 258, 1261, 1486,
2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or
target molecules but which do not interfere with binding of the 139, 258, 1261, 1486,
2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to
its target molecule can be derivatized to the wells of the plate, and unbound target or 139,

20   258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 protein trapped in the wells by antibody conjugation. Methods for detecting such
complexes, in addition to those described above for the GST-immobilized complexes,
include immunodetection of complexes using antibodies reactive with the 139, 258, 1261,
1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

25   protein or target molecule, as well as enzyme-linked assays which rely on detecting an
enzymatic activity associated with the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,
10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or target molecule.

In another embodiment, modulators of 139, 258, 1261, 1486, 2398, 2414, 7660,
8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression are identified in a

30   method wherein a cell is contacted with a candidate compound and the expression of 139,
258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 mRNA or protein in the cell is determined. The level of expression of 139, 258,
1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 mRNA or protein in the presence of the candidate compound is compared to the

level of expression of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

12680, 17921, 32248, 60489 or 93804 mRNA or protein in the absence of the candidate

compound. The candidate compound can then be identified as a modulator of 139, 258,

1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

5   93804 expression based on this comparison. For example, when expression of 139, 258,

1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804 mRNA or protein is greater (statistically significantly greater) in the presence of

the candidate compound than in its absence, the candidate compound is identified as a

stimulator of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

10   32248, 60489 or 93804 mRNA or protein expression. Alternatively, when expression of

139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

60489 or 93804 mRNA or protein is less (statistically significantly less) in the presence of

the candidate compound than in its absence, the candidate compound is identified as an

inhibitor of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

15   32248, 60489 or 93804 mRNA or protein expression. The level of 139, 258, 1261, 1486,

2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA or

protein expression in the cells can be determined by methods described herein for

detecting 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

32248, 60489 or 93804 mRNA or protein.

20   In yet another aspect of the invention, the 139, 258, 1261, 1486, 2398, 2414, 7660,

8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins can be used as "bait

proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317;

Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-

12054; Bartel et al. (1993) Biotechniques 14:920-924; Iwabuchi et al. (1993) Oncogene

25   8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact

with 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

60489 or 93804 ("139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

17921, 32248, 60489 or 93804 -binding proteins" or "139, 258, 1261, 1486, 2398, 2414,

7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -bp") and are involved

30   in 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

60489 or 93804 activity. Such 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

10550, 12680, 17921, 32248, 60489 or 93804 -binding proteins are also likely to be

involved in the propagation of signals by the 139, 258, 1261, 1486, 2398, 2414, 7660,

8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins or 139, 258, 1261,

1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804
targets as, for example, downstream elements of a 139, 258, 1261, 1486, 2398, 2414,
7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -mediated signaling
pathway. Alternatively, such 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,
10550, 12680, 17921, 32248, 60489 or 93804 -binding proteins are likely to be 139, 258,
1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 inhibitors.

The two-hybrid system is based on the modular nature of most transcription
factors, which consist of separable DNA-binding and activation domains. Briefly, the
assay utilizes two different DNA constructs. In one construct, the gene that codes for a
139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,
60489 or 93804 protein is fused to a gene encoding the DNA binding domain of a known
transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library
of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a
gene that codes for the activation domain of the known transcription factor. If the "bait"
and the "prey" proteins are able to interact, in vivo, forming a 139, 258, 1261, 1486, 2398,
2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -dependent
complex, the DNA-binding and activation domains of the transcription factor are brought
into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ)
which is operably linked to a transcriptional regulatory site responsive to the transcription
factor. Expression of the reporter gene can be detected and cell colonies containing the
functional transcription factor can be isolated and used to obtain the cloned gene which
encodes the protein which interacts with the 139, 258, 1261, 1486, 2398, 2414, 7660,
8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein.

In another aspect, the invention pertains to a combination of two or more of the
assays described herein. For example, a modulating agent can be identified using a cell-
based or a cell free assay, and the ability of the agent to modulate the activity of a 139,
258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 protein can be confirmed in vivo, e.g., in an animal such as an animal model for
cardiovascular disease, e.g., atherosclerosis and/or thrombosis, as described herein.

This invention further pertains to novel agents identified by the above-described
screening assays. Accordingly, it is within the scope of this invention to further use an
agent identified as described herein in an appropriate animal model. For example, an
agent identified as described herein (e.g., a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,

10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulating agent, an antisense 139,
258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 nucleic acid molecule, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,
10550, 12680, 17921, 32248, 60489 or 93804 -specific antibody, or a 139, 258, 1261,
5   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -
binding partner) can be used in an animal model to determine the efficacy, toxicity, or side
effects of treatment with such an agent. Alternatively, an agent identified as described
herein can be used in an animal model to determine the mechanism of action of such an
agent. Furthermore, this invention pertains to uses of novel agents identified by the above-
10   described screening assays for treatments as described herein.

Any of the compounds, including but not limited to compounds such as those
identified in the foregoing assay systems, may be tested for the ability to treat
cardiovascular disease symptoms. Cell-based and animal model-based assays for the
identification of compounds exhibiting such an ability to ameliorate cardiovascular disease
15   systems are described herein.

In one aspect, cell-based systems, as described herein, may be used to identify
compounds which may act to treat at least one cardiovascular disease symptom. For
example, such cell systems may be exposed to a compound, suspected of exhibiting an
ability to treat cardiovascular disease symptoms, at a sufficient concentration and for a
20   time sufficient to elicit such an amelioration of cardiovascular disease symptoms in the
exposed cells. After exposure, the cells are examined to determine whether one or more of
the cardiovascular disease cellular phenotypes has been altered to resemble a more normal
or more wild type, non-cardiovascular disease phenotype. Cellular phenotypes that are
associated with cardiovascular disease states include aberrant proliferation and migration,
25   angiogenesis, deposition of extracellular matrix components, accumulation of intracellular
lipids, and expression of growth factors, cytokines, and other inflammatory mediators.

In addition, animal-based cardiovascular disease systems, such as those described
herein, may be used to identify compounds capable of ameliorating cardiovascular disease
symptoms. Such animal models may be used as test substrates for the identification of
30   drugs, pharmaceuticals, therapies, and interventions which may be effective in treating
cardiovascular disease. For example, animal models may be exposed to a compound,
suspected of exhibiting an ability to ameliorate cardiovascular disease symptoms, at a
sufficient concentration and for a time sufficient to elicit such an amelioration of
cardiovascular disease symptoms in the exposed animals. The response of the animals to

the exposure may be monitored by assessing the reversal of disorders associated with cardiovascular disease, for example, by counting the number of atherosclerotic plaques and/or measuring their size before and after treatment.

With regard to intervention, any treatments which reverse any aspect of cardiovascular disease symptoms should be considered as candidates for human cardiovascular disease therapeutic intervention. Dosages of test agents may be determined by deriving dose-response curves.

Additionally, gene expression patterns may be utilized to assess the ability of a compound to ameliorate cardiovascular disease symptoms. For example, the expression pattern of one or more genes may form part of a "gene expression profile" or "transcriptional profile" which may be then be used in such an assessment. "Gene expression profile" or "transcriptional profile", as used herein, includes the pattern of mRNA expression obtained for a given tissue or cell type under a given set of conditions. Such conditions may include, but are not limited to, atherosclerosis, ischemia/reperfusion, hypertension, restenosis, and arterial inflammation, including any of the control or experimental conditions described herein, for example, atherogenic cytokine stimulation of macrophages. Gene expression profiles may be generated, for example, by utilizing a differential display procedure, Northern analysis and/or RT-PCR. In one embodiment, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences may be used as probes and/or PCR primers for the generation and corroboration of such gene expression profiles.

Gene expression profiles may be characterized for known states, either cardiovascular disease or normal, within the cell- and/or animal-based model systems. Subsequently, these known gene expression profiles may be compared to ascertain the effect a test compound has to modify such gene expression profiles, and to cause the profile to more closely resemble that of a more desirable profile.

For example, administration of a compound may cause the gene expression profile of a cardiovascular disease model system to more closely resemble the control system. Administration of a compound may, alternatively, cause the gene expression profile of a control system to begin to mimic a cardiovascular disease state. Such a compound may, for example, be used in further characterizing the compound of interest, or may be used in the generation of additional animal models.

II.   Cell- and Animal-Based Model Systems

Described herein are cell- and animal-based systems which act as models for cardiovascular disease. These systems may be used in a variety of applications. For example, the cell- and animal-based model systems may be used to further characterize

5   differentially expressed genes associated with cardiovascular disease, *e.g.*, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 . In addition, animal- and cell-based assays may be used as part of screening strategies . designed to identify compounds which are capable of ameliorating cardiovascular disease symptoms, as described, below. Thus, the animal- and cell-based models may be used to

10   identify drugs, pharmaceuticals, therapies and interventions which may be effective in treating cardiovascular disease. Furthermore, such animal models may be used to determine the LD50 and the ED50 in animal subjects, and such data can be used to determine the *in vivo* efficacy of potential cardiovascular disease treatments.

15   A. Animal-Based Systems

Animal-based model systems of cardiovascular disease may include, but are not limited to, non-recombinant and engineered transgenic animals.

Non-recombinant animal models for cardiovascular disease may include, for example, genetic models. Such genetic cardiovascular disease models may include, for

20   example, ApoB or ApoR deficient pigs (Rapacz, *et al.*, 1986, *Science* 234:1573-1577) and Watanabe heritable hyperlipidemic (WHHL) rabbits (Kita *et al.*, 1987, *Proc. Natl. Acad. Sci USA* 84: 5928-5931). Transgenic mouse models in cardiovascular disease and angiogenesis are reviewed in Carmeliet, P. and Collen, D. (2000) J. Pathol. 190:387-405.

Non-recombinant, non-genetic animal models of atherosclerosis may include, for

25   example, pig, rabbit, or rat models in which the animal has been exposed to either chemical wounding through dietary supplementation of LDL, or mechanical wounding through balloon catheter angioplasty. Animal models of cardiovascular disease also include rat myocardial infarction models (described in, for example, Schwarz, ER *et al.* (2000) *J. Am. Coll. Cardiol.* 35:1323-1330) and models of chromic cardiac ischemia in

30   rabbits (described in, for example, Operschall, C *et al.* (2000) *J. Appl. Physiol.* 88:1438-1445).

Additionally, animal models exhibiting cardiovascular disease symptoms may be engineered by using, for example, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences described above, in

conjunction with techniques for producing transgenic animals that are well known to those of skill in the art. For example, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences may be introduced into, and overexpressed in, the genome of the animal of interest, or, if endogenous 139, 258, 1261,

5   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences are present, they may either be overexpressed or, alternatively, be disrupted in order to underexpress or inactivate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression, such as described for the disruption of ApoE in mice (Plump et al., 1992, Cell 71: 343-353).

10   The host cells of the invention can also be used to produce non-human transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in

15   which exogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequences have been introduced into their genome or homologous recombinant animals in which endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequences have been altered. Such animals are useful for studying the function and/or activity of a 139, 258,

20   1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 and for identifying and/or evaluating modulators of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal

25   includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the

30   transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene has been altered by homologous recombination between the endogenous gene

and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal used in the methods of the invention can be created by introducing a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

5   17921, 32248, 60489 or 93804 -encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 cDNA sequence can be introduced as a transgene into the genome of a non-human animal. Alternatively, a

10   nonhuman homologue of a human 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, such as a mouse or rat 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, can be used as a transgene. Alternatively, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene homologue,

15   such as another 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 family member, can be isolated based on hybridization to the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 cDNA sequences and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of

20   expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 transgene to direct expression of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and

25   microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, both by Leder *et al.*, U.S. Patent No. 4,873,191 by Wagner *et al.* and in Hogan, B., *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal

30   can be identified based upon the presence of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 transgene in its genome and/or expression of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene.

Moreover, transgenic animals carrying a transgene encoding a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene. The 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene can be a human gene but more preferably, is a non-human homologue of a human 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene. For example, a rat 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene can be used to construct a homologous recombination nucleic acid molecule, *e.g.*, a vector, suitable for altering an endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene in the mouse genome. In a preferred embodiment, the homologous recombination nucleic acid molecule is designed such that, upon homologous recombination, the endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the homologous recombination nucleic acid molecule can be designed such that, upon homologous recombination, the endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein).

In the homologous recombination nucleic acid molecule, the altered portion of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene is flanked at its 5' and 3' ends by additional nucleic acid sequence of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene to allow for homologous recombination to occur between the exogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene carried by the homologous recombination nucleic acid molecule and an endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

32248, 60489 or 93804 gene in a cell, *e.g.*, an embryonic stem cell. The additional

flanking 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

32248, 60489 or 93804 nucleic acid sequence is of sufficient length for successful

homologous recombination with the endogenous gene. Typically, several kilobases of

5   flanking DNA (both at the 5' and 3' ends) are included in the homologous recombination

nucleic acid molecule (see, *e.g.*, Thomas, K.R. and Capecchi, M. R. (1987) *Cell* 51:503 for

a description of homologous recombination vectors). The homologous recombination

nucleic acid molecule is introduced into a cell, *e.g.*, an embryonic stem cell line (*e.g.*, by

electroporation) and cells in which the introduced 139, 258, 1261, 1486, 2398, 2414,

10   7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene has homologously

recombined with the endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

10550, 12680, 17921, 32248, 60489 or 93804 gene are selected (see *e.g.*, Li, E. *et al.*

(1992) *Cell* 69:915). The selected cells can then injected into a blastocyst of an animal

(*e.g.*, a mouse) to form aggregation chimeras (see *e.g.*, Bradley, A. in *Teratocarcinomas*

15   *and Embryonic Stem Cells: A Practical Approach*, E.J. Robertson, ed. (IRL, Oxford, 1987)

pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant

female foster animal and the embryo brought to term. Progeny harboring the

homologously recombined DNA in their germ cells can be used to breed animals in which

all cells of the animal contain the homologously recombined DNA by germline

20   transmission of the transgene. Methods for constructing homologous recombination

nucleic acid molecules, *e.g.*, vectors, or homologous recombinant animals are described

further in Bradley, A. (1991) *Current Opinion in Biotechnology* 2:823-829 and in PCT

International Publication Nos.: WO 90/11354 by Le Mouellec *et al.*; WO 91/01140 by

Smithies *et al.*; WO 92/0968 by Zijlstra *et al.*; and WO 93/04169 by Berns *et al.*

25   In another embodiment, transgenic non-human animals for use in the methods of

the invention can be produced which contain selected systems which allow for regulated

expression of the transgene. One example of such a system is the *cre/loxP* recombinase

system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, see,

e.g., Lakso *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a

30   recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae*

(O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used

to regulate expression of the transgene, animals containing transgenes encoding both the

Cre recombinase and a selected protein are required. Such animals can be provided

through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic

animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut, I. *et al.* (1997) *Nature* 385:810-813 and
5   PCT International Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it
10   develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

The 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 transgenic animals that express 139, 258, 1261, 1486, 2398,
15   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA or a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 peptide (detected immunocytochemically, using antibodies directed against 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 epitopes) at easily detectable levels should then be further evaluated to identify
20   those animals which display characteristic cardiovascular disease symptoms. Such cardiovascular disease symptoms may include, for example, increased prevalence and size of fatty streaks and/or cardiovascular disease plaques.

Additionally, specific cell types (*e.g.*, endothelial cells) within the transgenic animals may be analyzed and assayed for cellular phenotypes characteristic of
25   cardiovascular disease. In the case of endothelial cells, such phenotypes include, but are not limited to cell proliferation, migration, angiogenesis, production of proinflammatory growth factors and cytokines, and adhesion to inflammatory cells. In the case of monocytes, such phenotypes may include but are not limited to increases in rates of LDL uptake, adhesion to endothelial cells, transmigration, foam cell formation, fatty streak
30   formation, and production of foam cell specific products. Cellular phenotypes may include a particular cell type's pattern of expression of genes associated with cardiovascular disease as compared to known expression profiles of the particular cell type in animals exhibiting cardiovascular disease symptoms.

B. Cell-Based Systems

Cells that contain and express 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences which encode a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

5   93804 protein, and, further, exhibit cellular phenotypes associated with cardiovascular disease, may be used to identify compounds that exhibit anti-cardiovascular disease activity. Such cells may include non-recombinant monocyte cell lines, such as U937 (ATCC# CRL-1593), THP-1 (ATCC#TIB-202), and P388D1 (ATCC# TIB-63); endothelial cells such as human umbilical vein endothelial cells (HUVECs), human

10 microvascular endothelial cells (HMVEC), and bovine aortic endothelial cells (BAECs); as well as generic mammalian cell lines such as HeLa cells and COS cells, e.g., COS-7 (ATCC# CRL-1651). Further, such cells may include recombinant, transgenic cell lines. For example, the cardiovascular disease animal models of the invention, discussed above, may be used to generate cell lines, containing one or more cell types involved in

15 cardiovascular disease, that can be used as cell culture models for this disorder. While primary cultures derived from the cardiovascular disease transgenic animals of the invention may be utilized, the generation of continuous cell lines is preferred. For examples of techniques which may be used to derive a continuous cell line from the transgenic animals, see Small et al., (1985) Mol. Cell Biol. 5:642-648.

20 Alternatively, cells of a cell type known to be involved in cardiovascular disease may be transfected with sequences capable of increasing or decreasing the amount of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression within the cell. For example, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences may be

25 introduced into, and overexpressed in, the genome of the cell of interest, or, if endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences are present, they may be either overexpressed or, alternatively disrupted in order to underexpress or inactivate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression.

30 In order to overexpress a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, the coding portion of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene may be ligated to a regulatory sequence which is capable of driving gene expression in the cell type of interest, e.g., an endothelial cell. Such regulatory regions

will be well known to those of skill in the art, and may be utilized in the absence of undue experimentation. Recombinant methods for expressing target genes are described above.

For underexpression of an endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequence, such a

5   sequence may be isolated and engineered such that when reintroduced into the genome of the cell type of interest, the endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 alleles will be inactivated. Preferably, the engineered 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequence is introduced via gene targeting such that

10   the endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequence is disrupted upon integration of the engineered 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequence into the cell's genome. Transfection of host cells with 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

15   93804 genes is discussed, above.

Cells treated with compounds or transfected with 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 genes can be examined for phenotypes associated with cardiovascular disease. In the case of monocytes, such phenotypes include but are not limited to increases in rates of LDL

20   uptake, adhesion to endothelial cells, transmigration, foam cell formation, fatty streak formation, and production by foam cells of growth factors such as bFGF, IGF-I, VEGF, IL-1, M-CSF, TGFβ, TGFα, TNFα, HB-EGF, PDGF, IFN-γ, and GM-CSF. Transmigration rates, for example, may be measured using the in vitro system of Navab *et al.* (1988) *J. Clin. Invest.* 82:1853-1863, by quantifying the number of monocytes that

25   migrate across the endothelial monolayer and into the collagen layer of the subendothelial space.

Similarly, endothelial cells can be treated with test compounds or transfected with genetically engineered 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 genes. The endothelial cells can then be examined

30   for phenotypes associated with cardiovascular disease, including, but not limited to changes in cellular morphology, cell proliferation, cell migration, and mononuclear cell adhesion; or for the effects on production of other proteins involved in cardiovascular disease such as adhesion molecules (*e.g.*, ICAM, VCAM, E-selectin), growth factors and

cytokines (e.g., PDGF, IL-1β, TNFα, MCF), and proteins involved in angiogenesis (e.g., FLK, FLT).

Transfection of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid may be accomplished by using

5 standard techniques (described in, for example, Ausubel (1989) *supra*). Transfected cells should be evaluated for the presence of the recombinant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences, for expression and accumulation of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA, and for the presence of

10 recombinant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein production. In instances wherein a decrease in 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression is desired, standard techniques may be used to demonstrate whether a decrease in endogenous 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

15 10550, 12680, 17921, 32248, 60489 or 93804 gene expression and/or in 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein production is achieved.

Cellular models for the study of cardiovascular disease and angiogenesis include models of endothelial cell differentiation on Matrigel (Baatout, S. *et al.* (1996) *Rom. J.*

20 *Intern. Med.* 34:263-269; Benelli, R *et al.* (1999) *Int. J. Biol. Markers* 14:243-246), embryonic stem cell models of vascular morphogenesis (Doetschman, T. *et al.* (1993) *Hypertension* 22:618-629), the culture of microvessel fragments in physiological gels (Hoying, JB *et al.* (1996) *In Vitro Cell Dev. Biol. Anim.* 32: 409-419; US Patent No. 5,976,782), and the treatment of endothelial cells and smooth muscle cells with

25 atherogenic and angiogenic factors including growth factors and cytokines (e.g., IL-1β, PDGF, TNFα, VEGF), homocysteine, and LDL. *In vitro* angiogenesis models are described in, for example, Black, AF *et al.* (1999) *Cell Biol. Toxicol.* 15:81-90.

III.   Predictive Medicine:

30   The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

protein and/or nucleic acid expression as well as 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity, in the context of a biological sample (e.g., blood, serum, cells, e.g., endothelial cells, or tissue, e.g., vascular tissue) to thereby determine whether an individual is afflicted with a cardiovascular

5   disease. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a cardiovascular disorder. For example, mutations in a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene can be assayed for in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby phophylactically treat an

10   individual prior to the onset of a cardiovascular disorder, e.g., atherosclerosis.

Another aspect of the invention pertains to monitoring the influence of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulators (e.g., anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibodies or 139, 258, 1261, 1486, 2398,

15   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 ribozymes) on the expression or activity of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 in clinical trials.

These and other agents are described in further detail in the following sections.

20   A.   Diagnostic Assays For Cardiovascular Disease

To determine whether a subject is afflicted with a cardiovascular disease, a biological sample may be obtained from a subject and the biological sample may be contacted with a compound or an agent capable of detecting a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or

25   nucleic acid (e.g., mRNA or genomic DNA) that encodes a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, in the biological sample. A preferred agent for detecting 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to 139, 258, 1261, 1486, 2398,

30   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA or genomic DNA. The nucleic acid probe can be, for example, the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid set forth in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a portion thereof, such as an oligonucleotide of at least 15, 20, 25, 30, 25, 40, 45, 50, 100, 250 or

500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

5   A preferred agent for detecting 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein in a sample is an antibody capable of binding to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody,

10   or a fragment thereof (*e.g.*, Fab or F(ab')2) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody

15   using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

The term "biological sample" is intended to include tissues, cells, and biological fluids isolated from a subject, as well as tissues, cells, and fluids present within a subject. That is, the detection method of the invention can be used to detect 139, 258, 1261, 1486,

20   2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of 139, 258, 1261, 1486, 2398,

25   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of 139,

30   258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein include introducing into a subject a labeled anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, mRNA, or genomic DNA, such that the presence

5   of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, mRNA or genomic DNA in the control sample with the presence of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

10   12680, 17921, 32248, 60489 or 93804 protein, mRNA or genomic DNA in the test sample.

B.   Prognostic Assays For Cardiovascular Disease

The present invention further pertains to methods for identifying subjects having or

15   at risk of developing a cardiovascular disease associated with aberrant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity.

As used herein, the term "aberrant" includes a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity

20   which deviates from the wild type 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the wild type developmental pattern of expression or the subcellular pattern of expression. For example, aberrant 139, 258, 1261, 1486, 2398,

25   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity is intended to include the cases in which a mutation in the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene causes the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene to be under-expressed or over-expressed and situations in

30   which such mutations result in a non-functional 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or a protein which does not function in a wild-type fashion, e.g., a protein which does not interact with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804 substrate, or one which interacts with a non-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate.

The assays described herein, such as the preceding diagnostic assays or the following assays, can be used to identify a subject having or at risk of developing a

5   cardiovascular disease, e.g., including but not limited to, atherosclerosis, ischemia/reperfusion injury, hypertension, restenosis, arterial inflammation, and endothelial cell disorders. A biological sample may be obtained from a subject and tested for the presence or absence of a genetic alteration. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more

10   nucleotides from a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, 2) an addition of one or more nucleotides to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, 3) a substitution of one or more nucleotides of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, 4) a

15   chromosomal rearrangement of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, 5) an alteration in the level of a messenger RNA transcript of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, 6) aberrant modification of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

20   93804 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, 8) a non-wild type level of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -protein, 9) allelic loss of a 139, 258, 1261, 1486,

25   2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, and 10) inappropriate post-translational modification of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -protein.

As described herein, there are a large number of assays known in the art which can be used for detecting genetic alterations in a 139, 258, 1261, 1486, 2398, 2414, 7660,

30   8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene. For example, a genetic alteration in a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene may be detected using a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR)

(see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene (see Abravaya *et al.* (1995) *Nucleic*

5   *Acids Res.* 23:675-682). This method includes collecting a biological sample from a subject, isolating nucleic acid (*e.g.*, genomic DNA, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene under conditions such that hybridization and amplification of the

10 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for

15 detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or any other

20 nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a 139, 258, 1261, 1486, 2398, 2414,

25 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene from a biological sample can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and

30 control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be identified by

hybridizing biological sample derived and control nucleic acids, *e.g.*, DNA or RNA, to
high density arrays containing hundreds or thousands of oligonucleotide probes (Cronin,
M.T. *et al.* (1996) *Human Mutation* 7:244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine*
2:753-759). For example, genetic mutations in 139, 258, 1261, 1486, 2398, 2414, 7660,
5   8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be identified in two
dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et
al.* (1996) *supra.* Briefly, a first hybridization array of probes can be used to scan through
long stretches of DNA in a sample and control to identify base changes between the
sequences by making linear arrays of sequential, overlapping probes. This step allows for
10   the identification of point mutations. This step is followed by a second hybridization array
that allows for the characterization of specific mutations by using smaller, specialized
probe arrays complementary to all variants or mutations detected. Each mutation array is
composed of parallel probe sets, one complementary to the wild-type gene and the other
complementary to the mutant gene.

15   In yet another embodiment, any of a variety of sequencing reactions known in the
art can be used to directly sequence the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,
10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene in a biological sample and
detect mutations by comparing the sequence of the 139, 258, 1261, 1486, 2398, 2414,
7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 in the biological sample
20   with the corresponding wild-type (control) sequence. Examples of sequencing reactions
include those based on techniques developed by Maxam and Gilbert (1977) *Proc. Natl.
Acad. Sci. USA* 74:560) or Sanger (1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also
contemplated that any of a variety of automated sequencing procedures can be utilized
when performing the diagnostic assays (Naeve, C. W. (1995) *Biotechniques* 19:448-53),
25   including sequencing by mass spectrometry (see, *e.g.*, PCT International Publication No.
WO 94/16101; Cohen *et al.* (1996) *Adv. Chromatogr.* 36:127-162; and Griffin *et al.* (1993)
Appl. Biochem. Biotechnol. 38:147-159).

            Other methods for detecting mutations in the 139, 258, 1261, 1486, 2398, 2414,
7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene include methods
30   in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA
or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art
technique of "mismatch cleavage" starts by providing heteroduplexes formed by
hybridizing (labeled) RNA or DNA containing the wild-type 139, 258, 1261, 1486, 2398,
2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequence with

potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA

5   hybrids treated with S1 nuclease to enzymatically digest the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for

10   example, Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397 and Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA

15   mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662).

20   According to an exemplary embodiment, a probe based on a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequence, *e.g.*, a wild-type 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage

25   products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 genes. For example, single strand conformation

30   polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766; see also Cotton (1993) *Mutat. Res.* 285:125-144 and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay

5 may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment the movement of mutant or wild-type fragments in

10 polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to ensure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in

15 place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the

20 known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl Acad. Sci USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and

25 hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential

30 hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain

embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of

5   amplification.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, or small molecule) to effectively

10   treat a cardiovascular disease, *e.g.*, atherosclerosis.

C.   Monitoring of Effects During Clinical Trials

The present invention further provides methods for determining the effectiveness of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

15   60489 or 93804 modulator (*e.g.*, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator identified herein) in treating a cardiovascular disease, *e.g.*, atherosclerosis and/or thrombosis, in a subject. For example, the effectiveness of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator in increasing 139, 258, 1261, 1486,

20   2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression, protein levels, or in upregulating 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity, can be monitored in clinical trials of subjects exhibiting decreased 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression, protein

25   levels, or downregulated 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity. Alternatively, the effectiveness of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator in decreasing 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression, protein levels, or in

30   downregulating 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity, can be monitored in clinical trials of subjects exhibiting increased 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene expression, protein levels, or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity.

In such clinical trials, the expression or activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene, and preferably, other genes that have been implicated in, for example, atherosclerosis and/or thrombosis can be used as a "read out" or marker of the phenotype of a particular cell, *e.g.*, a vascular

5   endothelial cell.

For example, and not by way of limitation, genes, including 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 , that are modulated in cells by treatment with an agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity

10   (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents which modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity on subjects suffering from a cardiovascular disease in, for example, a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of 139, 258, 1261, 1486, 2398, 2414,

15   7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 and other genes implicated in the cardiovascular disease. The levels of gene expression (*e.g.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods described herein, or by measuring the levels of activity of 139, 258, 1261, 1486,

20   2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity. This response state may be determined before, and at various points during treatment of

25   the individual with the agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

30   activity (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, or small molecule identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, mRNA, or genomic

DNA in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, mRNA, or genomic DNA in the post-administration samples; (v)

5   comparing the level of expression or activity of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, mRNA, or genomic DNA in the pre-administration sample with the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the

10   administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease

15   expression or activity of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 to lower levels than detected, i.e. to decrease the effectiveness of the agent. According to such an embodiment, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity may be used as an indicator of the effectiveness of an agent, even in the absence of

20   an observable phenotypic response.

IV.   Methods of Treatment of Subjects Suffering From Cardiovascular Disease:

The present invention provides for both prophylactic and therapeutic methods of treating a subject, e.g., a human, at risk of (or susceptible to) a cardiovascular disease such

25   as atherosclerosis, ischemia/reperfusion injury, hypertension, restenosis, arterial inflammation, thrombosis, and endothelial cell disorders. With regard to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics," as used herein, refers to the application of genomics technologies

30   such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers to the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype").

Thus, another aspect of the invention provides methods for tailoring an subject's prophylactic or therapeutic treatment with either the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 molecules of the present invention or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

5   17921, 32248, 60489 or 93804 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

10 A.   Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a cardiovascular disease by administering to the subject an agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

15 12680, 17921, 32248, 60489 or 93804 activity, e.g., modulation of calcium influx, cellular migration, or formation of atherosclerotic lesions. Subjects at risk for a cardiovascular disease, e.g., atherosclerosis and/or thrombosis, can be identified by, for example, any or a combination of the diagnostic or prognostic assays described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of

20 aberrant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity, such that a cardiovascular disease is prevented or, alternatively, delayed in its progression. Depending on the type of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 aberrancy, for example, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

25 10550, 12680, 17921, 32248, 60489 or 93804 , 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 agonist or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

30

B. Therapeutic Methods

Described herein are methods and compositions whereby cardiovascular disease symptoms may be ameliorated. Certain cardiovascular diseases are brought about, at least in part, by an excessive level of a gene product, or by the presence of a gene product

exhibiting an abnormal or excessive activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of cardiovascular disease symptoms. Techniques for the reduction of gene expression levels or the activity of a protein are discussed below.

5   Alternatively, certain other cardiovascular diseases are brought about, at least in part, by the absence or reduction of the level of gene expression, or a reduction in the level of a protein's activity. As such, an increase in the level of gene expression and/or the activity of such proteins would bring about the amelioration of cardiovascular disease symptoms.

10   In some cases, the up-regulation of a gene in a disease state reflects a protective role for that gene product in responding to the disease condition. Enhancement of such a gene's expression, or the activity of the gene product, will reinforce the protective effect it exerts. Some cardiovascular disease states may result from an abnormally low level of · activity of such a protective gene. In these cases also, an increase in the level of gene
15   expression and/or the activity of such gene products would bring about the amelioration of cardiovascular disease symptoms. Techniques for increasing target gene expression levels or target gene product activity levels are discussed herein.

           Accordingly, another aspect of the invention pertains to methods of modulating 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,
20   60489 or 93804 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 or agent that modulates one or more of the activities of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
25   93804 protein activity associated with the cell (e.g., an endothelial cell or an ovarian cell). An agent that modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,
30   60489 or 93804 protein (e.g., a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 ligand or substrate), a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibody, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 agonist or antagonist, a peptidomimetic of a 139, 258, 1261,

1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 agonist or antagonist, or other small molecule. In one embodiment, the agent stimulates one or more 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activities. Examples of such stimulatory agents include active

5   139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein and a nucleic acid molecule encoding 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 that has been introduced into the cell. In another embodiment, the agent inhibits one or more 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

10   93804 activities. Examples of such inhibitory agents include antisense 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid molecules, anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibodies, and 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 inhibitors. These

15   modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

20   protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity. In another embodiment, the method involves administering a 139, 258, 1261,

25   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity.

Stimulation of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

30   12680, 17921, 32248, 60489 or 93804 activity is desirable in situations in which 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 is abnormally downregulated and/or in which increased 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity is likely to have a beneficial effect. Likewise, inhibition of 139, 258, 1261, 1486, 2398,

2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   activity is
desirable in situations in which 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,
10550, 12680, 17921,.32248, 60489 or 93804   is abnormally upregulated and/or in which
decreased 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,
5   32248, 60489 or 93804   activity is likely to have a beneficial effect.

(i) Methods for Inhibiting Target Gene Expression, Synthesis, or Activity

As discussed above, genes involved in cardiovascular disorders may cause such
disorders via an increased level of gene activity. In some cases, such up-regulation may
10   have a causative or exacerbating effect on the disease state. A variety of techniques may
be used to inhibit the expression, synthesis, or activity of such genes and/or proteins.

For example, compounds such as those identified through assays described above,
which exhibit inhibitory activity, may be used in accordance with the invention to
ameliorate cardiovascular disease symptoms. Such molecules may include, but are not
15   limited to, small organic molecules, peptides, antibodies, and the like.

For example, compounds can be administered that compete with endogenous
ligand for the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,
17921, 32248, 60489 or 93804   protein. The resulting reduction in the amount of ligand-
bound 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,
20   32248, 60489 or 93804   protein will modulate endothelial cell physiology. Compounds
that can be particularly useful for this purpose include, for example, soluble proteins or
peptides, such as peptides comprising one or more of the extracellular domains, or portions
and/or analogs thereof, of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,
10550, 12680, 17921, 32248, 60489 or 93804   protein, including, for example, soluble
25   fusion proteins such as Ig-tailed fusion proteins. (For a discussion of the production of Ig-
tailed fusion proteins, see, for example, U.S. Pat. No. 5,116,964). Alternatively,
compounds, such as ligand analogs or antibodies, that bind to the 139, 258, 1261, 1486,
2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   receptor
site, but do not activate the protein, (e.g., receptor-ligand antagonists) can be effective in
30   inhibiting 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,
32248, 60489 or 93804   protein activity.

Further, antisense and ribozyme molecules which inhibit expression of the 139,
258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804   gene may also be used in accordance with the invention to inhibit aberrant 139,

258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene activity. Still further, triple helix molecules may be utilized in inhibiting aberrant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene activity.

5   The antisense nucleic acid molecules used in the methods of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation.

10   The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention include direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can

15   be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells

20   using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, an antisense nucleic acid molecule used in the methods of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid

25   molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

30   In still another embodiment, an antisense nucleic acid used in the methods of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes

(described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to

catalytically cleave 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

17921, 32248, 60489 or 93804 mRNA transcripts to thereby inhibit translation of 139,

258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

5   93804 mRNA. A ribozyme having specificity for a 139, 258, 1261, 1486, 2398, 2414,

7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -encoding nucleic acid

can be designed based upon the nucleotide sequence of a 139, 258, 1261, 1486, 2398,

2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 cDNA disclosed

herein (*i.e.*, SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29). For

10   example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the

nucleotide sequence of the active site is complementary to the nucleotide sequence to be

cleaved in a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

32248, 60489 or 93804 -encoding mRNA (see, for example, Cech *et al.* U.S. Patent No.

4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742). Alternatively, 139, 258, 1261,

15   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 .

mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a

pool of RNA molecules (see, for example, Bartel, D. and Szostak, J.W. (1993) *Science*

261:1411-1418).

139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

20   32248, 60489 or 93804 gene expression can also be inhibited by targeting nucleotide

sequences complementary to the regulatory region of the 139, 258, 1261, 1486, 2398,

2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 (*e.g.*, the 139,

258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804 promoter and/or enhancers) to form triple helical structures that prevent

25   transcription of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

17921, 32248, 60489 or 93804 gene in target cells (see, for example, Helene, C. (1991)

Anticancer Drug Des. 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-

36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15).

Antibodies that are both specific for the 139, 258, 1261, 1486, 2398, 2414, 7660,

30   8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein and interfere with its

activity may also be used to modulate or inhibit 139, 258, 1261, 1486, 2398, 2414, 7660,

8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein function. Such

antibodies may be generated using standard techniques described herein, against the 139,

258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804   protein itself or against peptides corresponding to portions of the protein. Such
antibodies include but are not limited to polyclonal, monoclonal, Fab fragments, single
chain antibodies, or chimeric antibodies.

In instances where the target gene protein is intracellular and whole antibodies are
used, internalizing antibodies may be preferred. Lipofectin liposomes may be used to
deliver the antibody or a fragment of the Fab region which binds to the target epitope into
cells. Where fragments of the antibody are used, the smallest inhibitory
fragment which binds to the target protein's binding domain is preferred. For example,
peptides having an amino acid sequence corresponding to the domain of the variable
region of the antibody that binds to the target gene protein may be used. Such peptides
may be synthesized chemically or produced via recombinant DNA technology using
methods well known in the art (described in, for example, Creighton (1983), *supra*; and
Sambrook *et al.* (1989) *supra*). Single chain neutralizing antibodies which bind to
intracellular target gene epitopes may also be administered. Such single chain antibodies
may be administered, for example, by expressing nucleotide sequences encoding single-
chain antibodies within the target cell population by utilizing, for example, techniques
such as those described in Marasco *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-
7893).

In some instances, the target gene protein is extracellular, or is a transmembrane
protein, such as the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,
17921, 32248, 60489 or 93804   protein. Antibodies that are specific for one or more
extracellular domains of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,
12680, 17921, 32248, 60489 or 93804   protein, for example, and that interfere with its
activity, are particularly useful in treating cardiovascular disease. Such antibodies are
especially efficient because they can access the target domains directly from the
bloodstream. Any of the administration techniques described below which are appropriate
for peptide administration may be utilized to effectively administer inhibitory target gene
antibodies to their site of action.

(ii) Methods for Restoring or Enhancing Target Gene Activity

Genes that cause cardiovascular disease may be underexpressed within
cardiovascular disease situations. Alternatively, the activity of the protein products of
such genes may be decreased, leading to the development of cardiovascular disease

symptoms. Such down-regulation of gene expression or decrease of protein activity might have a causative or exacerbating effect on the disease state.

In some cases, genes that are up-regulated in the disease state might be exerting a protective effect. A variety of techniques may be used to increase the expression,

5   synthesis, or activity of genes and/or proteins that exert a protective effect in response to cardiovascular disease conditions.

Described in this section are methods whereby the level 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   activity may be increased to levels wherein cardiovascular disease symptoms are ameliorated. The

10   level of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   activity may be increased, for example, by either increasing the level of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   gene expression or by increasing the level of active 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

15   93804   protein which is present.

For example, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   protein, at a level sufficient to ameliorate cardiovascular disease symptoms may be administered to a patient exhibiting such symptoms. Any of the techniques discussed below may be used for such administration.

20   One of skill in the art will readily know how to determine the concentration of effective, non-toxic doses of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   protein, utilizing techniques such as those described below.

Additionally, RNA sequences encoding a 139, 258, 1261, 1486, 2398, 2414, 7660,

25   8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   protein may be directly administered to a patient exhibiting cardiovascular disease symptoms, at a concentration sufficient to produce a level of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   protein such that cardiovascular disease symptoms are ameliorated. Any of the techniques discussed below, which achieve

30   intracellular administration of compounds, such as, for example, liposome administration, may be used for the administration of such RNA molecules. The RNA molecules may be produced, for example, by recombinant techniques such as those described herein.

Further, subjects may be treated by gene replacement therapy. One or more copies of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

60489 or 93804 gene, or a portion thereof, that directs the production of a normal 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein with 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 function, may be inserted into cells using vectors which

5   include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes. Additionally, techniques such as those described above may be used for the introduction of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene sequences into human cells.

10   Cells, preferably, autologous cells, containing 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expressing gene sequences may then be introduced or reintroduced into the subject at positions which allow for the amelioration of cardiovascular disease symptoms. Such cell replacement techniques may be preferred, for example, when the gene product is a secreted,

15   extracellular gene product.

        C.   Pharmaceutical Compositions
        Another aspect of the invention pertains to methods for treating a subject suffering from a cardiovascular disease, _e.g._, atherosclerosis. These methods involve administering

20   to a subject an agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity (_e.g._, an agent identified by a screening assay described herein), or a combination of such agents. In another embodiment, the method involves administering to a subject a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

25   protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 expression or activity.
        Stimulation of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity is desirable in situations in which 139, 258,

30   1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 is abnormally downregulated and/or in which increased 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity is likely to have a beneficial effect. Likewise, inhibition of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity is

desirable in situations in which 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 is abnormally upregulated and/or in which decreased 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity is likely to have a beneficial effect, *e.g.*, inhibition of atherosclerotic lesion formation.

The agents which modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity can be administered to a subject using pharmaceutical compositions suitable for such administration. Such compositions typically comprise the agent (*e.g.*, nucleic acid molecule, protein, or antibody) and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition used in the therapeutic methods of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™

(BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a

5   solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can

10   be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays

15   absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the agent that modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity (*e.g.*, a fragment of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or an anti-139,

20   258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other

25   ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can

30   be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible

binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid,

5   Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant,

10   e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic

15   acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The agents that modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity can also be prepared in the form of

20   suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the agents that modulate 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity are prepared with carriers that will protect the compound against rapid elimination from the body, such

25   as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova

30   Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage

unit form for ease of administration and uniformity of dosage. Dosage unit form as used

herein refers to physically discrete units suited as unitary dosages for the subject to be

treated; each unit containing a predetermined quantity of active compound calculated to

5   produce the desired therapeutic effect in association with the required pharmaceutical

carrier. The specification for the dosage unit forms of the invention are dictated by and

directly dependent on the unique characteristics of the agent that modulates 139, 258,

1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804 activity and the particular therapeutic effect to be achieved, and the limitations

10   inherent in the art of compounding such an agent for the treatment of subjects.

Toxicity and therapeutic efficacy of such agents can be determined by standard

pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining

the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically

effective in 50% of the population). The dose ratio between toxic and therapeutic effects is

15   the therapeutic index and can be expressed as the ratio LD50/ED50. Agents which exhibit

large therapeutic indices are preferred. While agents that exhibit toxic side effects may be

used, care should be taken to design a delivery system that targets such agents to the site of

affected tissue in order to minimize potential damage to uninfected cells and, thereby,

reduce side effects.

20   The data obtained from the cell culture assays and animal studies can be used in

formulating a range of dosage for use in humans. The dosage of such 139, 258, 1261,

1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

modulating agents lies preferably within a range of circulating concentrations that include

the ED50 with little or no toxicity. The dosage may vary within this range depending upon

25   the dosage form employed and the route of administration utilized. For any agent used in

the therapeutic methods of the invention, the therapeutically effective dose can be

estimated initially from cell culture assays. A dose may be formulated in animal models to

achieve a circulating plasma concentration range that includes the IC50 (i.e., the

concentration of the test compound which achieves a half-maximal inhibition of

30   symptoms) as determined in cell culture. Such information can be used to more accurately

determine useful doses in humans. Levels in plasma may be measured, for example, by

high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (i.e.,

an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about

0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

In a preferred example, a subject is treated with antibody, protein, or polypeptide in the range of between about 0.1 to 20 mg/kg body weight, one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. It will also be appreciated that the effective dosage of antibody, protein, or polypeptide used for treatment may increase or decrease over the course of a particular treatment. Changes in dosage may result and become apparent from the results of diagnostic assays as described herein.

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds. It is understood that appropriate doses of small molecule agents depends upon a number of factors within the ken of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of the small molecule will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the small molecule to have upon the nucleic acid or polypeptide of the invention.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about

500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated.

5   Such appropriate doses may be determined using the assays described herein. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In

10   addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

15   Further, an antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy

20   anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan,

25   carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and

30   vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A,

pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),

5   granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-

10   56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The

15   Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982). Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as

20   described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules used in the methods of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see, e.g., Chen et al. (1994) Proc. Natl. Acad. Sci.

25   USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene

30   delivery system.

D.   Pharmacogenomics

In conjunction with the therapeutic methods of the invention, pharmacogenomics (i.e., the study of the relationship between a subject's genotype and that subject's response

to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant

5   pharmacogenomics studies in determining whether to administer an agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity, as well as tailoring the dosage and/or therapeutic regimen of treatment with an agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity.

10   Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp.Pharmacol. Physiol.* 23(10-11): 983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a

15   single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate aminopeptidase deficiency (G6PD) is a common inherited enzymopathy in which the main

20   clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene

25   marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants). Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be

30   generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map

based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

5   Alternatively, a method termed the "candidate gene approach" can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug target is known (e.g., a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein used in the methods of the present invention), all common variants of that gene can be fairly easily identified in the

10   population and it can be determined if having one version of the gene versus another is associated with a particular drug response.

        As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2)

15   and the cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among

20   different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as

25   demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

        Alternatively, a method termed the "gene expression profiling" can be utilized to

30   identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 molecule or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator used in the

methods of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for

5   prophylactic or therapeutic treatment of a subject. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and, thus, enhance therapeutic or prophylactic efficiency when treating a subject suffering from a cardiovascular disease, e.g., atherosclerosis, with an agent which modulates 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

10   93804 activity.

V.   Recombinant Expression Vectors and Host Cells Used in the Methods of the
      Invention

      The methods of the invention (e.g., the screening assays described herein) include

15   the use of vectors, preferably expression vectors, containing a nucleic acid encoding a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop

20   into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the

25   genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and

30   "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors to be used in the methods of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression,

5 which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory

10 sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel (1990) *Methods Enzymol.* 185:3-7. Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells and those which direct expression of the nucleotide sequence only in certain host

15 cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as

20 described herein (*e.g.*, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins, mutant forms of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins, fusion proteins, and the like).

The recombinant expression vectors to be used in the methods of the invention can

25 be designed for expression of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins in prokaryotic or eukaryotic cells. For example, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host

30 cells are discussed further in Goeddel (1990) *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either

fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein
encoded therein, usually to the amino terminus of the recombinant protein. Such fusion
vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2)
to increase the solubility of the recombinant protein; and 3) to aid in the purification of the

5   recombinant protein by acting as a ligand in affinity purification. Often, in fusion
expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion
moiety and the recombinant protein to enable separation of the recombinant protein from
the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and
their cognate recognition sequences, include Factor Xa, thrombin and enterokinase.

10   Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and
Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and
pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E
binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be utilized in 139, 258, 1261, 1486, 2398, 2414,

15   7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity assays, (*e.g.*,
direct assays or competitive assays described in detail below), or to generate antibodies
specific for 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,
32248, 60489 or 93804 proteins. In a preferred embodiment, a 139, 258, 1261, 1486,
2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fusion

20   protein expressed in a retroviral expression vector of the present invention can be utilized
to infect bone marrow cells which are subsequently transplanted into irradiated recipients.
The pathology of the subject recipient is then examined after sufficient time has passed
(*e.g.*, six weeks).

In another embodiment, a nucleic acid of the invention is expressed in mammalian

25   cells using a mammalian expression vector. Examples of mammalian expression vectors
include pCDM8 (Seed, B. (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987)
EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control
functions are often provided by viral regulatory elements. For example, commonly used
promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus

30   40. For other suitable expression systems for both prokaryotic and eukaryotic cells see
chapters 16 and 17 of Sambrook, J. *et al.*, *Molecular Cloning: A Laboratory Manual. 2nd
ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring
Harbor, NY, 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid).

5   The methods of the invention may further use a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA. Regulatory sequences

10   operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or

15   attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

20   Another aspect of the invention pertains to the use of host cells into which a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid molecule of the invention is introduced, *e.g.*, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid molecule within a recombinant expression vector or a 139, 258, 1261, 1486, 2398,

25   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain

30   modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

A host cell used in the methods of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. Accordingly, the invention further provides methods for producing a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of the invention (into which a recombinant expression vector encoding a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein has been introduced) in a suitable medium such that a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein is produced. In another embodiment, the method further comprises isolating a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein from the medium or the host cell.

VI.   Isolated Nucleic Acid Molecules Used in the Methods of the Invention

The methods of the invention include the use of isolated nucleic acid molecules that encode 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins or biologically active portions thereof, as well as nucleic acid fragments sufficient for use as hybridization probes to identify 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -encoding nucleic acid molecules (e.g., 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA) and fragments for use as PCR

primers for the amplification or mutation of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.,* cDNA or genomic DNA) and RNA molecules (*e.g.,* mRNA) and analogs of the DNA or

5 RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

A nucleic acid molecule used in the methods of the present invention, *e.g.,* a nucleic acid molecule having the nucleotide sequence of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a portion thereof, can be isolated using standard molecular

10 biology techniques and the sequence information provided herein. Using all or portion of the nucleic acid sequence of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, as a hybridization probe, 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (*e.g.,* as described in Sambrook, J., Fritsh, E. F., and

15 Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 can be isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide primers designed based upon the sequence of SEQ

20 ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29.

A nucleic acid used in the methods of the invention can be amplified using cDNA, mRNA or, alternatively, genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. Furthermore, oligonucleotides corresponding to 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

25 10550, 12680, 17921, 32248, 60489 or 93804 nucleotide sequences can be prepared by standard synthetic techniques, *e.g.,* using an automated DNA synthesizer.

In a preferred embodiment, the isolated nucleic acid molecules used in the methods of the invention comprise the nucleotide sequence shown in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, a complement of the nucleotide sequence shown in SEQ ID

30 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a portion of any of these nucleotide sequences. A nucleic acid molecule which is complementary to the nucleotide sequence shown in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, is one which is sufficiently complementary to the nucleotide sequence shown in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 such that it can hybridize to the nucleotide sequence

shown in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 thereby forming a stable duplex.

In still another preferred embodiment, an isolated nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is at least about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to the entire length of the nucleotide sequence shown in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a portion of any of this nucleotide sequence.

Moreover, the nucleic acid molecules used in the methods of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, , for example, a fragment which can be used as a probe or primer or a fragment encoding a portion of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, *e.g.*, a biologically active portion of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense sequence of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, of an anti-sense sequence of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or of a naturally occurring allelic variant or mutant of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, . In one embodiment, a nucleic acid molecule used in the methods of the present invention comprises a nucleotide sequence which is greater than 100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, .

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4 and 6. Additional stringent conditions can

be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring
Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9 and 11. A preferred, non-
limiting example of stringent hybridization conditions includes hybridization in 4X sodium
chloride/sodium citrate (SSC), at about 65-70°C (or hybridization in 4X SSC plus 50%

5   formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°
C. A preferred, non-limiting example of highly stringent hybridization conditions includes
hybridization in 1X SSC, at about 65-70°C (or hybridization in 1X SSC plus 50%
formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-
70°C. A preferred, non-limiting example of reduced stringency hybridization conditions

10 includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X
SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC,
at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at
42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is
0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC

15 (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers;
washes are performed for 15 minutes each after hybridization is complete. The
hybridization temperature for hybrids anticipated to be less than 50 base pairs in length
should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is
determined according to the following equations. For hybrids less than 18 base pairs in

20 length, $T_m(°C) = 2(\# \text{ of } A + T \text{ bases}) + 4(\# \text{ of } G + C \text{ bases})$. For hybrids between 18 and
49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N)$, where
N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the
hybridization buffer ($[Na^+]$ for 1xSSC = 0.165 M). It will also be recognized by the
skilled practitioner that additional reagents may be added to hybridization and/or wash

25 buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for
example, nitrocellulose or nylon membranes, including but not limited to blocking agents
(*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating
agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in
particular, an additional preferred, non-limiting example of stringent hybridization

30 conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by
one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C, see *e.g.*, Church and Gilbert
(1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995, (or alternatively 0.2X SSC, 1% SDS).

In preferred embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a 139, 258, 1261, 1486, 2398, 2414, 7660,

5   8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, such as by measuring a level of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA levels or determining whether a genomic 139, 258, 1261,

10   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene has been mutated or deleted.

The methods of the invention further encompass the use of nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, due to degeneracy of the genetic code and thus encode the same 139,

15   258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins as those encoded by the nucleotide sequence shown in SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, . In another embodiment, an isolated nucleic acid molecule included in the methods of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO: NO:2, 4, 6, 8, 10, 12, 14,

20   16, 18, 20, 22, 24, 26, 28, 30.

The methods of the invention further include the use of allelic variants of human 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 , *e.g.*, functional and non-functional allelic variants. Functional allelic variants are naturally occurring amino acid sequence variants of the human 139, 258,

25   1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein that maintain a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, or substitution, deletion or insertion

30   of non-critical residues in non-critical regions of the protein.

Non-functional allelic variants are naturally occurring amino acid sequence variants of the human 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein that do not have a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity.

Non-functional allelic variants will typically contain a non-conservative substitution, deletion, or insertion or premature truncation of the amino acid sequence of SEQ ID NO: NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, or a substitution, insertion or deletion in critical residues or critical regions of the protein.

5   The methods of the present invention may further use non-human orthologues of the human 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. Orthologues of the human 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein are proteins that are isolated from non-human organisms and possess the same 139, 258, 1261,

10   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity.

        The methods of the present invention further include the use of nucleic acid molecules comprising the nucleotide sequence of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a portion thereof, in which a mutation has been introduced. The

15   mutation may lead to amino acid substitutions at "non-essential" amino acid residues or at "essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 (*e.g.*, the sequence of SEQ ID NO:2) without altering the biological activity, whereas an "essential" amino acid residue is

20   required for biological activity. For example, amino acid residues that are conserved among the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins of the present invention and other members of the family are not likely to be amenable to alteration.

        Mutations can be introduced into SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23,

25   25, 27, 29, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been

30   defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and

aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another

5   embodiment, mutations can be introduced randomly along all or part of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 biological activity to identify mutants that retain activity.

10   Following mutagenesis of SEQ ID 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, the encoded protein can be expressed recombinantly and the activity of the protein can be determined using the assay described herein.

Another aspect of the invention pertains to the use of isolated nucleic acid molecules which are antisense to the nucleotide sequence of SEQ ID 1, 3, 5, 7, 9, 11, 13,

15   15, 17, 19, 21, 23, 25, 27, 29, . An "antisense" nucleic acid comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary

20   to an entire 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 . The term "coding region" refers to the

25   region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 . The term "noncoding region" refers to 5' and 3' sequences which flank the coding

30   region that are not translated into amino acids (also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson

and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 139, 258, 1261,

5   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50

10   nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the

15   physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-

20   thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-

25   methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense

30   nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest). Antisense nucleic acid molecules used in the methods of the invention are further described above, in section IV.

In yet another embodiment, the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,
10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid molecules used in the
methods of the present invention can be modified at the base moiety, sugar moiety or
phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the
5   molecule. For example, the deoxyribose phosphate backbone of the nucleic acid
molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996)
Bioorganic & Medicinal Chemistry 4 (1): 5-23). As used herein, the terms "peptide
nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the
deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the
10   four natural nucleobases are retained. The neutral backbone of PNAs has been shown to
allow for specific hybridization to DNA and RNA under conditions of low ionic strength.
The synthesis of PNA oligomers can be performed using standard solid phase peptide
synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.*
(1996) *Proc. Natl. Acad. Sci.* 93:14670-675.

15   PNAs of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,
17921, 32248, 60489 or 93804 nucleic acid molecules can be used in the therapeutic and
diagnostic applications described herein. For example, PNAs can be used as antisense or
antigene agents for sequence-specific modulation of gene expression by, for example,
inducing transcription or translation arrest or inhibiting replication. PNAs of 139, 258,
20   1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 nucleic acid molecules can also be used in the analysis of single base pair
mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction
enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. *et
al.* (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B.
25   *et al.* (1996) *supra*; Perry-O'Keefe *et al.* (1996) *supra*).

In another embodiment, PNAs of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,
10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be modified, (*e.g.*, to enhance
their stability or cellular uptake), by attaching lipophilic or other helper groups to PNA, by
the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of
30   drug delivery known in the art. For example, PNA-DNA chimeras of 139, 258, 1261,
1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804
nucleic acid molecules can be generated which may combine the advantageous properties
of PNA and DNA. Such chimeras allow DNA recognition enzymes, (*e.g.*, RNAse H and
DNA polymerases), to interact with the DNA portion while the PNA portion would

provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup B. *et al.* (1996) *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup B. *et al.* (1996) *supra* and Finn

5   P.J. *et al.* (1996) *Nucleic Acids Res.* 24 (17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a between the PNA and the 5' end of DNA (Mag, M. *et al.* (1989) *Nucleic Acid Res.* 17: 5973-88). PNA monomers are then coupled in a stepwise

10   manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn P.J. *et al.* (1996) *supra*). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser, K.H. *et al.* (1975) *Bioorganic Med. Chem. Lett.* 5: 1119-11124).

In other embodiments, the oligonucleotide used in the methods of the invention

15   may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO88/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134). In addition, oligonucleotides can be modified

20   with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

25

VII.   Isolated 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,
        17921, 32248, 60489 or 93804 Proteins and Anti-139, 258, 1261, 1486, 2398,
        2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804
        Antibodies Used in the Methods of the Invention

30   The methods of the invention include the use of isolated 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibodies. In one embodiment, native 139, 258,

1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 proteins can be isolated from cells or tissue sources by an appropriate purification
scheme using standard protein purification techniques. In another embodiment, 139, 258,
1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
5   93804 proteins are produced by recombinant DNA techniques. Alternative to
recombinant expression, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,·
12680, 17921, 32248, 60489 or 93804 protein or polypeptide can be synthesized
chemically using standard peptide synthesis techniques.

As used herein, a "biologically active portion" of a 139, 258, 1261, 1486, 2398,
10   2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein includes
a fragment of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,
17921, 32248, 60489 or 93804 protein having a 139, 258, 1261, 1486, 2398, 2414, 7660,
8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity. Biologically active
portions of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,
15   32248, 60489 or 93804 protein include peptides comprising amino acid sequences
sufficiently identical to or derived from the amino acid sequence of the 139, 258, 1261,
1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804
protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, which include fewer amino
acids than the full length 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,
20   12680, 17921, 32248, 60489 or 93804 proteins, and exhibit at least one activity of a 139,
258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
93804 protein. Typically, biologically active portions comprise a domain or motif with at
least one activity of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,
12680, 17921, 32248, 60489 or 93804 protein (*e.g.*, the N-terminal region of the 139, 258,
25   1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or
·93804 protein that is believed to be involved in the regulation of apoptotic activity). A
biologically active portion of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,
10550, 12680, 17921, 32248, 60489 or 93804 protein can be a polypeptide which is, for
example, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300 or more amino acids in length.
30   Biologically active portions of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,
10550, 12680, 17921, 32248, 60489 or 93804 protein can be used as targets for
developing agents which modulate a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,
10183, 10550, 12680, 17921, 32248, 60489 or 93804 activity.

In a preferred embodiment, the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein used in the methods of the invention has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

5   60489 or 93804 protein is substantially identical to SEQ ID NO: NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and retains the functional activity of the protein of SEQ ID NO: NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail in subsection V above. Accordingly, in another embodiment, the 139, 258, 1261, 1486,

10   2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein used in the methods of the invention is a protein which comprises an amino acid sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2.

To determine the percent identity of two amino acid sequences or of two nucleic

15   acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least

20   50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, or 90% of the length of the reference sequence (e.g., when aligning a second sequence to the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 amino acid sequence of SEQ ID NO:2 having 500 amino acid residues, at least 75, preferably at least 150, more preferably at least 225, even more preferably at least

25   300, and even more preferably at least 400 or more amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or

30   nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

The comparison of sequences and determination of percent identity between two

sequences can be accomplished using a mathematical algorithm. In a preferred

embodiment, the percent identity between two amino acid sequences is determined using

the Needleman and Wunsch (*J. Mol. Biol.* 48:444-453 (1970)) algorithm which has been

5   incorporated into the GAP program in the GCG software package (available at ·

http://www.gcg.com), using either a Blosum 62 matrix or a PAM250 matrix, and a gap

weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another

preferred embodiment, the percent identity between two nucleotide sequences is

determined using the GAP program in the GCG software package (available at

10   http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60,

70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent

identity between two amino acid or nucleotide sequences is determined using the

algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.* 4:11-17 (1988)) which has

been incorporated into the ALIGN program (version 2.0 or 2.0U), using a PAM120 weight

15   residue table, a gap length penalty of 12 and a gap penalty of 4.

The methods of the invention may also use 139, 258, 1261, 1486, 2398, 2414,

7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 chimeric or fusion .

proteins. As used herein, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

12680, 17921, 32248, 60489 or 93804 "chimeric protein" or "fusion protein" comprises a

20   139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

60489 or 93804 polypeptide operatively linked to a non-139, 258, 1261, 1486, 2398,

2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide. An

"139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

60489 or 93804 polypeptide" refers to a polypeptide having an amino acid sequence   ·

25   corresponding to a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

17921, 32248, 60489 or 93804 molecule, whereas a "non-139, 258, 1261, 1486, 2398,

2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide"

refers to a polypeptide having an amino acid sequence corresponding to a protein which is

not substantially homologous to the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

30 · 10550, 12680, 17921, 32248, 60489 or 93804 protein, *e.g.*, a protein which is different

from the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,   ·

32248, 60489 or 93804 protein and which is derived from the same or a different

organism. Within a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

17921, 32248, 60489 or 93804 fusion protein the 139, 258, 1261, 1486, 2398, 2414,

7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide can correspond to all or a portion of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. In a preferred embodiment, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

5   93804 fusion protein comprises at least one biologically active portion of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. In another preferred embodiment, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fusion protein comprises at least two biologically active portions of a 139, 258, 1261, 1486, 2398, 2414,

10   7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide and the non-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide are fused in-frame to each other. The

15   non-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide can be fused to the N-terminus or C-terminus of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide.

For example, in one embodiment, the fusion protein is a GST-139, 258, 1261,

20   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fusion protein in which the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

25   93804.

In another embodiment, this fusion protein is a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,

30   10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be increased through use of a heterologous signal sequence.

The 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fusion proteins used in the methods of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The

139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fusion proteins can be used to affect the bioavailability of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate. Use of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein; (ii) mis-regulation of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 gene; and (iii) aberrant post-translational modification of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein.

Moreover, the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -fusion proteins used in the methods of the invention can be used as immunogens to produce anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibodies in a subject, to purify 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 ligands and in screening assays to identify molecules which inhibit the interaction of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 substrate.

Preferably, a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 chimeric or fusion protein used in the methods of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially

available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 - encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

5   10550, 12680, 17921, 32248, 60489 or 93804 protein.

The present invention also pertains to the use of variants of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins which function as either 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 agonists (mimetics) or as 139, 258, 1261,

10   1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antagonists. Variants of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. An agonist of the 139, 258,

15   1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. An antagonist of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

20   protein can inhibit one or more of the activities of the naturally occurring form of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein by, for example, competitively modulating a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 -mediated activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

25   32248, 60489 or 93804 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

30   12680, 17921, 32248, 60489 or 93804 protein.

In one embodiment, variants of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein which function as either 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 agonists (mimetics) or as 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,

10183, 10550, 12680, 17921, 32248, 60489 or 93804 antagonists can be identified by

screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a 139, 258, 1261,

1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804

protein for 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921,

5   32248, 60489 or 93804 protein agonist or antagonist activity. In one embodiment, a

variegated library of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

17921, 32248, 60489 or 93804 variants is generated by combinatorial mutagenesis at the

nucleic acid level and is encoded by a variegated gene library. A variegated library of 139,

258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

10   93804 variants can be produced by, for example, enzymatically ligating a mixture of

synthetic oligonucleotides into gene sequences such that a degenerate set of potential 139,

258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

93804 sequences is expressible as individual polypeptides, or alternatively, as a set of

larger fusion proteins (*e.g.*, for phage display) containing the set of 139, 258, 1261, 1486,

15   2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 sequences

therein. There are a variety of methods which can be used to produce libraries of potential

139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248,

60489 or 93804 variants from a degenerate oligonucleotide sequence. Chemical synthesis

of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the

20   synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set

of genes allows for the provision, in one mixture, of all of the sequences encoding the

desired set of potential 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

12680, 17921, 32248, 60489 or 93804 sequences. Methods for synthesizing degenerate

oligonucleotides are known in the art (see, *e.g.*, Narang, S.A. (1983) *Tetrahedron* 39:3;

25   Itakura *et al.* (1984) *Annu. Rev. Biochem.* 53:323; Itakura *et al.* (1984) *Science* 198:1056;

Ike *et al.* (1983) *Nucleic Acid Res.* 11:477).

        In addition, libraries of fragments of a 139, 258, 1261, 1486, 2398, 2414, 7660,

8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein coding sequence can

be used to generate a variegated population of 139, 258, 1261, 1486, 2398, 2414, 7660,

30   8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 fragments for screening and

subsequent selection of variants of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587,

10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. In one embodiment, a

library of coding sequence fragments can be generated by treating a double stranded PCR

fragment of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,

17921, 32248, 60489 or 93804 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal and internal fragments of various sizes of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

The methods of the present invention further include the use of anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibodies. An isolated 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 using standard techniques for polyclonal and monoclonal antibody preparation. A full-length 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein can be used or, alternatively, antigenic peptide fragments of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be used as immunogens. The antigenic peptide of 139, 258, 1261, 1486, 2398, 2414,

7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 comprises at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO: NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 and encompasses an epitope of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 such that an antibody raised against the peptide forms a specific immune complex with the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Preferred epitopes encompassed by the antigenic peptide are regions of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 that are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity.

A 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 immunogen is typically used to prepare antibodies by immunizing a suitable subject, (*e.g.*, rabbit, goat, mouse, or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein or a chemically synthesized 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 preparation induces a polyclonal anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibody response.

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 . Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183,

10550, 12680, 17921, 32248, 60489 or 93804 molecules. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of 139, 258, 1261, 1486, 2398, 2414, 7660,

5   8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 . A monoclonal antibody composition thus typically displays a single binding affinity for a particular 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein with which it immunoreacts.

    Polyclonal anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,

10   12680, 17921, 32248, 60489 or 93804 antibodies can be prepared as described above by immunizing a suitable subject with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 immunogen. The anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibody titer in the immunized subject can be monitored over time by standard

15   techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 . If desired, the antibody molecules directed against 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 can be isolated from the mammal (*e.g.*, from the blood) and further purified by well

20   known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, *e.g.*, when the anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally

25   described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also, Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci. USA* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal*

30   *Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R. H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); Lerner, E. A. (1981) *Yale J. Biol. Med.* 54:387-402; Gefter, M. L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an

immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma

5   producing a monoclonal antibody that binds 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 .

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or

10   93804 monoclonal antibody (see, *e.g.*, G. Galfre *et al.* (1977) *Nature* 266:55052; Gefter *et al.* (1977) *supra*; Lerner (1981) *supra*; and Kenneth (1980) *supra*). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (*e.g.*, a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine

15   hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard

20   techniques, *e.g.*, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after

25   several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 , *e.g.*, using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal

30   anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 to thereby isolate immunoglobulin library members that bind 139, 258, 1261, 1486,

2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 . Kits for
generating and screening phage display libraries are commercially available (*e.g.*, the
Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the
Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples

5   of methods and reagents particularly amenable for use in generating and screening
antibody display library can be found in, for example, Ladner *et al.* U.S. Patent No.
5,223,409; Kang *et al.* PCT International Publication No. WO 92/18619; Dower *et al.* PCT
International Publication No. WO 91/17271; Winter *et al.* PCT International Publication
WO 92/20791; Markland *et al.* PCT International Publication No. WO 92/15679; Breitling

10 *et al.* PCT International Publication WO 93/01288; McCafferty *et al.* PCT International
Publication No. WO 92/01047; Garrard *et al.* PCT International Publication No. WO
92/09690; Ladner *et al.* PCT International Publication No. WO 90/02809; Fuchs *et al.*
(1991) *Bio/Technology* 9:1370-1372; Hay *et al.* (1992) *Hum. Antibod. Hybridomas* 3:81-
85; Huse *et al.* (1989) *Science* 246:1275-1281; Griffiths *et al.* (1993) *EMBO J* 12:725-734;

15 Hawkins *et al.* (1992) *J. Mol. Biol.* 226:889-896; Clarkson *et al.* (1991) *Nature* 352:624-
628; Gram *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3576-3580; Garrad *et al.* (1991)
Bio/Technology 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc. Acid Res.* 19:4133-4137;
Barbas *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:7978-7982; and McCafferty *et al.*
(1990) *Nature* 348:552-554.

20   Additionally, recombinant anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587,
10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibodies, such as chimeric and
humanized monoclonal antibodies, comprising both human and non-human portions,
which can be made using standard recombinant DNA techniques, are within the scope of
the methods of the invention. Such chimeric and humanized monoclonal antibodies can be

25 produced by recombinant DNA techniques known in the art, for example using methods
described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.*
European Patent Application 184,187; Taniguchi, M., European Patent Application
171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT
International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567;

30 Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-
1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J.
Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218;
Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449;
Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science*

229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539;

Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and

Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

An anti-139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,
17921, 32248, 60489 or 93804 antibody can be used to detect 139, 258, 1261, 1486, 2398,
2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 protein (*e.g.*, in a
cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of
expression of the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680,
17921, 32248, 60489 or 93804 protein. Anti-139, 258, 1261, 1486, 2398, 2414, 7660,
8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 antibodies can be used
diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*,
to, for example, determine the efficacy of a given treatment regimen. Detection can be
facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance.
Examples of detectable substances include various enzymes, prosthetic groups, fluorescent
materials, luminescent materials, bioluminescent materials, and radioactive materials.
Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-
galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes
include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials
include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine,
dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a
luminescent material includes luminol; examples of bioluminescent materials include
luciferase, luciferin, and aequorin, and examples of suitable radioactive material include
^{125}I, ^{131}I, ^{35}S or ^{3}H.

This invention is further illustrated by the following examples which should not be
construed as limiting. The contents of all references, patents and published patent
applications cited throughout this application, as well as the Figure and the Sequence
Listing is incorporated herein by reference.

EXAMPLES

EXAMPLE 1:   **TISSUE DISTRIBUTION OF USING TAQMAN™ ANALYSIS**

This example describes the TaqMan™ procedure. The Taqman™ procedure is a
quantitative, reverse transcription PCR-based approach for detecting mRNA. The RT-

PCR reaction exploits the 5' nuclease activity of AmpliTaq Gold™ DNA Polymerase to cleave a TaqMan™ probe during PCR. Briefly, cDNA was generated from the samples of interest, *e.g.*, heart, kidney, liver, skeletal muscle, and various vessels, and used as the starting material for PCR amplification. In addition to the 5' and 3' gene-specific primers,

5   a gene-specific oligonucleotide probe (complementary to the region being amplified) was included in the reaction (*i.e.*, the Taqman™ probe). The TaqMan™ probe includes the oligonucleotide with a fluorescent reporter dye covalently linked to the 5' end of the probe (such as FAM (6-carboxyfluorescein), TET (6-carboxy-4,7,2',7'-tetrachlorofluorescein), JOE (6-carboxy-4,5-dichloro-2,7-dimethoxyfluorescein), or VIC) and a quencher dye

10   (TAMRA (6-carboxy-N,N,N',N'-tetramethylrhodamine) at the 3' end of the probe.

During the PCR reaction, cleavage of the probe separates the reporter dye and the quencher dye, resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in

15   suppression of the reporter fluorescence. During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites. The 5'-3' nucleolytic activity of the AmpliTaq™ Gold DNA Polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are then displaced from the target, and polymerization of the strand continues.

20   The 3' end of the probe is blocked to prevent extension of the probe during PCR. This process occurs in every cycle and does not interfere with the exponential accumulation of product. RNA was prepared using the trizol method and treated with DNase to remove contaminating genomic DNA. cDNA was synthesized using standard techniques. Mock cDNA synthesis in the absence of reverse transcriptase resulted in samples with no

25   detectable PCR amplification of the control gene confirms efficient removal of genomic DNA contamination.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than

30   routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed:

1.   A method for identifying a compound capable of treating a cardiovascular disorder, comprising assaying the ability of the compound to modulate 139,
5   258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid expression or 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide activity, thereby identifying a compound capable of treating a cardiovascular disorder.

10   2.   A method for identifying a compound capable of modulating lipid production comprising:
        a) contacting a cell which expresses 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 with a test compound; and

15   b) assaying the ability of the test compound to modulate the expression of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid or the activity of a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 polypeptide, thereby identifying a compound capable of modulating lipid production.

20

3.   A method for modulating lipid production in a cell comprising contacting a cell with a 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator, thereby modulating lipid production in the cell.

25

4.   The method of claim 2, wherein the cell is a hepatic cell.

5.   The method of claim 3, wherein the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator is a
30   small organic molecule, peptide, antibody or antisense nucleic acid molecule.

6.   The method of claim 3, wherein the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator is capable of modulating 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550,
35   12680, 17921, 32248, 60489 or 93804 polypeptide activity.

7.   The method of claim 6, wherein the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   modulator is a small organic molecule, peptide, antibody or antisense nucleic acid molecule.

5   8.   The method of claim 6, wherein the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   modulator is capable of modulating 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   nucleic acid expression.

10   9.   A method for treating a subject having a cardiovascular disorder characterized by aberrant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   polypeptide activity or aberrant 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 nucleic acid expression comprising administering to the subject a 139, 258, 1261, 1486, 15   2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 modulator, thereby treating said subject having a cardiovascular disorder.

10.   The method of claim 9, wherein said cardiovascular disorder is selected from the group consisting of arteriosclerosis, atherosclerosis, cardiac hypertrophy, 20   ischemia reperfusion injury, restenosis, arterial inflammation, vascular wall remodeling, ventricular remodeling, rapid ventricular pacing, coronary microembolism, tachycardia, bradycardia, pressure overload, aortic bending, coronary artery ligation, vascular heart disease, valvular disease, including but not limited to, valvular degeneration caused by calcification, rheumatic heart disease, endocarditis, or complications of artificial valves; 25   atrial fibrillation, long-QT syndrome, congestive heart failure, sinus node dysfunction, angina, heart failure, hypertension, atrial fibrillation, atrial flutter, pericardial disease, including but not limited to, pericardial effusion and pericarditis; cardiomyopathies, e.g., dilated cardiomyopathy or idiopathic cardiomyopathy, myocardial infarction, coronary artery disease, coronary artery spasm, ischemic disease, arrhythmia, sudden cardiac death, 30   and cardiovascular developmental disorders

11.   The method of claim 9, wherein said 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   modulator is administered in a pharmaceutically acceptable formulation.

35

12.   The method of claim 9, wherein the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   modulator is a small organic molecule, peptide, antibody or antisense nucleic acid molecule.

13.   The method of claim 9, wherein the 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   modulator is capable of modulating 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804   polypeptide activity.

SEQUENCE LISTING

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      Galvin, Katherine
      Chun, Miyoung

<120> Methods and Compositions to treat
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10183, 10550, 12680, 17921, 32248, 60489 OR 93804

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ctttatcatc ctggagaaca tctttgtctt gctgaccatt tggaaaacca agaaattcca 480
ccgacccatg tactatttta ttggcaatct ggccctctca gacctgttgg caggagtagc 540
ctacacagct aacctgctct tgtctggggc caccacctac aagctcactc ccgcccagtg 600
gtttctgcgg gaagggagta tgtttgtggc cctgtcagcc tccgtgttca gtctcctcgc 660
catcgccatt gagcgctata tcacaatgct gaaaatgaaa ctccacaacg ggagcaataa 720
cttccgcctc ttcctgctaa tcagcgcctg ctgggtcatc tccctcatcc tgggtggcct 780
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cgtcattctg tactgcagaa tctactcctt ggtcaggact cggagccgcc gcctgacgtt 960
ccgcaagaac atttccaagg ccagccgcag ctctgagaat gtggcgctgc tcaagaccgt 1020
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gcgtcgggcc ttcatccgga tcatgtcctg ctgcaagtgc ccgagcggag actctgctgg 1260
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gctgcaagcc agaggggaga agggggagaa tacgaacagc ctggtggtgt cgggtgttgg 1560
tgggtagagt tagttcctgt gaacaatgca ctgggaaggg tggagatcag gtcccggcct 1620
ggaatatata ttctaccccc ctggagcttt gattttgcac tgagccaaag gtctagcatt 1680
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cgtctctttg tctggagctt tgaggagatg ttttccttca ctttagtttc aaacccaagt 1800
gagtgtgtgc acttctgctt ctttagggat gcccgtgtaca tcccacaccc caccctccct 1860
tcccttcata ccccctcctca acgttctttt actttatact ttaactacct gagagttatc 1920
agagctgggg ttgtggaatg atcgatcatc tatagcaaat aggctatgtt gagtacgtag 1980
gctgtgggaa gatgaagatg gtttggaggt gtaaaacaat gtccttcgct gaggccaaag 2040
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aaaacatctt ttcaatgaaa tgtgttacca tttcatatcc attgaagccg aaatctgcat 2160
aaggaagccc actttatcta aatgatatta gccaggatcc ttggtgtcct aggagaaaca 2220
gacaagcaaa acaaagtgaa aaccgaatgg attaactttt gcaaccaag ggagatttct 2280

```
tagcaaatga gtctaacaaa tatgacatcc gtcttcccca ctttttgttga tgtttatttc 2340
agaatcttgt gtgattcatt tcaagcaaca acatgttgta ttttgttgtg ttaaaagtac 2400
ttttcttgat ttttgaatgt atttgtttca ggaagaagtc attttatgga ttttttctaac 2460
ccgtgttaac ttttctagaa tccacccctct tgtgcccttatt agcattactt taactggtag 2520
ggaacgccag aacttttaag tccagctatt cattagatag taattgaaga tatgtataaa 2580
tattacaaag aataaaaata tattactgtc tctttagtat ggttttcagt gcaattaaac 2640
cgagagatgt cttgttttttt taaaagaat agtatttaat aggtttctga cttttgtgga 2700
tcattttgca catagcttta tcaactttta aacattaata aactgatttt tttaaag    2757
```

<210> 2
<211> 381
<212> PRT
<213> Homo Sapien

<400> 2

```
Met Gly Pro Thr Ser Val Pro Leu Val Lys Ala His Arg Ser Ser Val
 1               5                  10                  15
Ser Asp Tyr Val Asn Tyr Asp Ile Ile Val Arg His Tyr Asn Tyr Thr
            20                  25                  30
Gly Lys Leu Asn Ile Ser Ala Asp Lys Glu Asn Ser Ile Lys Leu Thr
        35                  40                  45
Ser Val Val Phe Ile Leu Ile Cys Cys Phe Ile Ile Leu Glu Asn Ile
    50                  55                  60
Phe Val Leu Leu Thr Ile Trp Lys Thr Lys Lys Phe His Arg Pro Met
65                  70                  75                  80
Tyr Tyr Phe Ile Gly Asn Leu Ala Leu Ser Asp Leu Leu Ala Gly Val
                85                  90                  95
Ala Tyr Thr Ala Asn Leu Leu Leu Ser Gly Ala Thr Thr Tyr Lys Leu
            100                 105                 110
Thr Pro Ala Gln Trp Phe Leu Arg Glu Gly Ser Met Phe Val Ala Leu
        115                 120                 125
Ser Ala Ser Val Phe Ser Leu Leu Ala Ile Ala Ile Glu Arg Tyr Ile
    130                 135                 140
Thr Met Leu Lys Met Lys Leu His Asn Gly Ser Asn Asn Phe Arg Leu
145                 150                 155                 160
Phe Leu Leu Ile Ser Ala Cys Trp Val Ile Ser Leu Ile Leu Gly Gly
                165                 170                 175
Leu Pro Ile Met Gly Trp Asn Cys Ile Ser Ala Leu Ser Ser Cys Ser
            180                 185                 190
Thr Val Leu Pro Leu Tyr His Lys His Tyr Ile Leu Phe Cys Thr Thr
        195                 200                 205
Val Phe Thr Leu Leu Leu Leu Ser Ile Val Ile Leu Tyr Cys Arg Ile
    210                 215                 220
Tyr Ser Leu Val Arg Thr Arg Ser Arg Arg Leu Thr Phe Arg Lys Asn
225                 230                 235                 240
Ile Ser Lys Ala Ser Arg Ser Ser Glu Asn Val Ala Leu Leu Lys Thr
                245                 250                 255
Val Ile Ile Val Leu Ser Val Phe Ile Ala Cys Trp Ala Pro Leu Phe
            260                 265                 270
Ile Leu Leu Leu Leu Asp Val Gly Cys Lys Val Lys Thr Cys Asp Ile
        275                 280                 285
Leu Phe Arg Ala Glu Tyr Phe Leu Val Leu Ala Val Leu Asn Ser Gly
        290                 295                 300
Thr Asn Pro Ile Ile Tyr Thr Leu Thr Asn Lys Glu Met Arg Arg Ala
305                 310                 315                 320
Phe Ile Arg Ile Met Ser Cys Cys Lys Cys Pro Ser Gly Asp Ser Ala
                325                 330                 335
Gly Lys Phe Lys Arg Pro Ile Ile Ala Gly Met Glu Phe Ser Arg Ser
            340                 345                 350
Lys Ser Asp Asn Ser Ser His Pro Gln Lys Asp Glu Gly Asp Asn Pro
        355                 360                 365
```

```
Glu Thr Ile Met Ser Ser Gly Asn Val Asn Ser Ser Ser
    370             375             380
```

<210> 3
<211> 1472
<212> DNA
<213> Homo Sapien

<400> 3

```
ggcgcgaggg ctggagctcc gaagagggcc gagtaggagc tgcatggaca gcatgcgtct 60
ctccgccggt cccgacgcgg ggccctcggg caactccagc ccatggtggc ctctggccac 120
cggcgctggc aacacaagcc gggaggccga agccctcggg gagggcaacg gcccaccgag 180
ggacgtgcgc aacgaggagc tggccaaact ggagatcgcc gtgctggcgg tgactttcgc 240
ggtggccgtg ctgggcaaca gcagcgtact gctggctctg caccggacgc cgcgcaagac 300
gtcccgcatg cacctcttca tccgacacct cagcctggcc gacctggccg tggcattctt 360
ccaggtgctg ccgcaaatgt gctgggacat cacctaccgc ttccgcggcc ccgactggct 420
gtgccgcgtg gtgaagcacc tgcaggtgtt cggcatgttt gcgtcggcct acatgctggt 480
agtcatgaca gccgaccgct acatcgcggt gtgccaccg ctcaagactc tgcaacagcc 540
cgcgcgccgc tcgcgcctca tgatcgcggc cgcctgggtg ctgagcttcg tgctgagcac 600
gccgcagtac ttcgtcttct ccatgatcga ggtgaacaat gtcaccaagg cccgcgactg 660
ctgggccacc ttcatccagc cctggggttc tcgtgcctac gtgacctgga tgacgggcgg 720
catctttgtg gcgcccgtgg tcatcttggg tacctgctac ggcttcatct gctacaacat 780
ctggtgcaac gtccgcggga agacggcgtc gcgccagagc aagggtgcag agcaagcggg 840
tgtggccttc caaaagggt tcctgctcgc accctgtgtc agcagcgtga gtccatttc 900
ccgggccaag atccgcacgg tgaagatgac ttttgtgatc gtgacggctt acatcgtctg 960
ctgggcgcct ttcttcatca tccagatgtg gtctgtctgg gatcccatgt ccgtctggac 1020
cgaatcggaa aaccctacca tcaccatcac tgcattactg ggttccttga atagctgctg 1080
taatccctgg atatacatgt tttttagtgg ccatctcctt caagactgtg ttcaaagctt 1140
cccatgctgc aaaacatga aggaaaaatt caacaagaa gatactgaca gtatgagcag 1200
aagacagact tttttattcta acaatcgaag cccaacaaac agtacgggta gtggaagga 1260
ctcgcctaaa tcttccaagt ccatcaaatt cattcctgtt tcaacttgag ccttgcattc 1320
atgcaacttg attcttgtga ttgactttt ggctcattag ctgaattgag ctagaaatca 1380
caagaacaaa tacactttat taatataacc ataaatcaat tcattgtgta tgagactgtg 1440
tttctagttg catttcatat tgctaccaaa cc                               1472
```

<210> 4
<211> 418
<212> PRT
<213> Homo Sapien

<400> 4

```
Met Arg Leu Ser Ala Gly Pro Asp Ala Gly Pro Ser Gly Asn Ser Ser
1               5                   10                  15
Pro Trp Trp Pro Leu Ala Thr Gly Ala Gly Asn Thr Ser Arg Glu Ala
            20                  25                  30
Glu Ala Leu Gly Glu Gly Asn Gly Pro Pro Arg Asp Val Arg Asn Glu
        35                  40                  45
Glu Leu Ala Lys Leu Glu Ile Ala Val Leu Ala Val Thr Phe Ala Val
    50                  55                  60
Ala Val Leu Gly Asn Ser Ser Val Leu Leu Ala Leu His Arg Thr Pro
65                  70                  75                  80
Arg Lys Thr Ser Arg Met His Leu Phe Ile Arg His Leu Ser Leu Ala
                85                  90                  95
Asp Leu Ala Val Ala Phe Phe Gln Val Leu Pro Gln Met Cys Trp Asp
            100                 105                 110
Ile Thr Tyr Arg Phe Arg Gly Pro Asp Trp Leu Cys Arg Val Val Lys
        115                 120                 125
His Leu Gln Val Phe Gly Met Phe Ala Ser Ala Tyr Met Leu Val Val
    130                 135                 140
Met Thr Ala Asp Arg Tyr Ile Ala Val Cys His Pro Leu Lys Thr Leu
```

```
145                    150                    155                    160
Gln Gln Pro Ala Arg Arg Ser Arg Leu Met Ile Ala Ala Ala Trp Val
                 165                    170                    175
Leu Ser Phe Val Leu Ser Thr Pro Gln Tyr Phe Val Phe Ser Met Ile
             180                    185                    190
Glu Val Asn Asn Val Thr Lys Ala Arg Asp Cys Trp Ala Thr Phe Ile
         195                    200                    205
Gln Pro Trp Gly Ser Arg Ala Tyr Val Thr Trp Met Thr Gly Gly Ile
     210                    215                    220
Phe Val Ala Pro Val Val Ile Leu Gly Thr Cys Tyr Gly Phe Ile Cys
225                    230                    235                    240
Tyr Asn Ile Trp Cys Asn Val Arg Gly Lys Thr Ala Ser Arg Gln Ser
                 245                    250                    255
Lys Gly Ala Glu Gln Ala Gly Val Ala Phe Gln Lys Gly Phe Leu Leu
             260                    265                    270
Ala Pro Cys Val Ser Ser Val Lys Ser Ile Ser Arg Ala Lys Ile Arg
         275                    280                    285
Thr Val Lys Met Thr Phe Val Ile Val Thr Ala Tyr Ile Val Cys Trp
     290                    295                    300
Ala Pro Phe Phe Ile Ile Gln Met Trp Ser Val Trp Asp Pro Met Ser
305                    310                    315                    320
Val Trp Thr Glu Ser Glu Asn Pro Thr Ile Thr Ile Thr Ala Leu Leu
                 325                    330                    335
Gly Ser Leu Asn Ser Cys Cys Asn Pro Trp Ile Tyr Met Phe Phe Ser
             340                    345                    350
Gly His Leu Leu Gln Asp Cys Val Gln Ser Phe Pro Cys Cys Gln Asn
         355                    360                    365
Met Lys Glu Lys Phe Asn Lys Glu Asp Thr Asp Ser Met Ser Arg Arg
     370                    375                    380
Gln Thr Phe Tyr Ser Asn Asn Arg Ser Pro Thr Asn Ser Thr Gly Met
385                    390                    395                    400
Trp Lys Asp Ser Pro Lys Ser Ser Lys Ser Ile Lys Phe Ile Pro Val
                 405                    410                    415
Ser Thr
```

```
<210> 5
<211> 2481
<212> DNA
<213> Homo Sapien

<400> 5
cgaacttggg gggagtgcac agaagaactt cgggagcgca cgcgggacca gggaccaggc 60
tgagactcgg ggcgccagtc cgggcagggg cagcgggagc cggccggaga tgccctgtat 120
ccaagcccaa tatgggacac cagcaccgag tccgggaccc cgtgaccacc tggcaagcga 180
cccctgacc cctgagttca tcaagcccac catggacctg ccagccccg aggcagcccc 240
cgctgccccc actgccctgc ccagcttcag caccttcatg acggctaca caggagagtt 300
tgacaccttc ctctaccagc tgccaggaac agtccagcca tgctcctcag cctcctcctc 360
ggcctcctcc acatcctcgt cctcagccac ctccctgcc tctgcttcct tcaagttcga 420
ggacttccag gtgtacggct gctaccccgg ccccctgagc ggcccagtgg atgaggccct 480
gtcctccagt ggctctgact actatggcag cccctgctcg gccccgtcgc cctccacgcc 540
cagcttccag ccgccccagc tctctccctg ggatggctcc ttcggccact tctcgcccag 600
ccagacttac gaaggcctgc gggcatggac agagcagctg cccaaagcct ctgggccccc 660
acagcctcca gccttctttt ccttcagtcc tcccaccggc cccagcccca gcctggccca 720
gagccccctg aagttgttcc cctcacaggc cacccaccag ctggggagg gagagagcta 780
ttccatgcct acggccttcc caggtttggc acccacttct ccacaccttg agggctcggg 840
gatactggat acacccgtga cctcaaccaa ggcccggagc ggggccccag tggaagtga 900
aggccgctgt gctgtgtgtg gggacaacgc ttcatgccag cattatggtg tccgcacatg 960
tgagggctgc aagggcttct tcaagcgcac agtgcagaaa aacgccaagt acatctgcct 1020
ggctaacaag gactgccctg tggacaagag gcggcgaaac cgctgccagt tctgccgctt 1080
```

```
ccagaagtgc ctggcggtgg gcatggtgaa ggaagttgtc cgaacagaca gcctgaaggg 1140
gcggcggggc cggctacctt caaaacccaa gcagccccca gatgcctccc ctgccaatct 1200
cctcacttcc ctggtccgtg cacacctgga ctcagggccc agcactgcca aactggacta 1260
ctccaagttc caggagctgg tgctgcccca ctttgggaag gaagatgctg gggatgtaca 1320
gcagttctac gacctgctct ccggttctct ggaggtcatc cgcaagtggg cggagaagat 1380
ccctggcttt gctgagctgt caccggctga ccaggacctg ttgctggagt cggccttcct 1440
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ctgcctctct gcccttgtcc tcatcaccga ccggcatggg ctgcaggagc gcggcgggt 1680
ggaggagctg cagaaccgca tcgccagctg cctgaaggag cacgtggcag ctgtggcggg 1740
cgagccccag ccagccagct gcctgtcacg tctgttgggc aaactgcccg agctgcggac 1800
cctgtgcacc cagggcctgc agcgcatctt ctacctcaag ctggaggact tggtgccccc 1860
tccacccatc attgacaaga tcttcatgga cacgctgccc ttctgacccc tgcctgcctg 1920
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caaagggggat gaagggaggg aggctcaagg cccttggggg aggggggatgc cttcatgggg 2100
gtgacccacg atgtgttctt atcccccccg cctggccacc ggcctttatg ttttttgtaa 2160
gataaaccgt ttttaacaca tagcgccgtg ctgtaaataa gcccagtact gctgtaaata 2220
caggaagaaa gagcttgagg tgggagcggg ctgggaggaa gggatgggcc ccggccttcc 2280
tgggcagcct ttccagcctc ctgctgggct ctctcttcct accctccttc cacatgtaca 2340
tgtacataaa ctgtcactct aggaagaaga caaatgacag attctgacca tttatatttg 2400
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aaaaaataga catgtagttg g                                           2481
```

<210> 6
<211> 598
<212> PRT
<213> Homo Sapien

<400> 6

```
Met Pro Cys Ile Gln Ala Gln Tyr Gly Thr Pro Ala Pro Ser Pro Gly
  1               5                  10                  15
Pro Arg Asp His Leu Ala Ser Asp Pro Leu Thr Pro Glu Phe Ile Lys
             20                  25                  30
Pro Thr Met Asp Leu Ala Ser Pro Glu Ala Ala Pro Ala Ala Pro Thr
         35                  40                  45
Ala Leu Pro Ser Phe Ser Thr Phe Met Asp Gly Tyr Thr Gly Glu Phe
     50                  55                  60
Asp Thr Phe Leu Tyr Gln Leu Pro Gly Thr Val Gln Pro Cys Ser Ser
 65                  70                  75                  80
Ala Ser Ser Ser Ala Ser Ser Thr Ser Ser Ser Ser Ala Thr Ser Pro
                 85                  90                  95
Ala Ser Ala Ser Phe Lys Phe Glu Asp Phe Gln Val Tyr Gly Cys Tyr
                100                 105                 110
Pro Gly Pro Leu Ser Gly Pro Val Asp Glu Ala Leu Ser Ser Ser Gly
            115                 120                 125
Ser Asp Tyr Tyr Gly Ser Pro Cys Ser Ala Pro Ser Pro Ser Thr Pro
        130                 135                 140
Ser Phe Gln Pro Pro Gln Leu Ser Pro Trp Asp Gly Ser Phe Gly His
145                 150                 155                 160
Phe Ser Pro Ser Gln Thr Tyr Glu Gly Leu Arg Ala Trp Thr Glu Gln
                165                 170                 175
Leu Pro Lys Ala Ser Gly Pro Pro Gln Pro Ala Phe Phe Ser Phe
                180                 185                 190
Ser Pro Pro Thr Gly Pro Ser Pro Ser Leu Ala Gln Ser Pro Leu Lys
            195                 200                 205
Leu Phe Pro Ser Gln Ala Thr His Gln Leu Gly Glu Gly Glu Ser Tyr
        210                 215                 220
Ser Met Pro Thr Ala Phe Pro Gly Leu Ala Pro Thr Ser Pro His Leu
225                 230                 235                 240
```

```
Glu Gly Ser Gly Ile Leu Asp Thr Pro Val Thr Ser Thr Lys Ala Arg
             245             250             255
Ser Gly Ala Pro Gly Gly Ser Glu Gly Arg Cys Ala Val Cys Gly Asp
             260             265             270
Asn Ala Ser Cys Gln His Tyr Gly Val Arg Thr Cys Glu Gly Cys Lys
             275             280             285
Gly Phe Phe Lys Arg Thr Val Gln Lys Asn Ala Lys Tyr Ile Cys Leu
             290             295             300
Ala Asn Lys Asp Cys Pro Val Asp Lys Arg Arg Arg Asn Arg Cys Gln
305             310             315             320
Phe Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Val Lys Glu Val
             325             330             335
Val Arg Thr Asp Ser Leu Lys Gly Arg Arg Gly Arg Leu Pro Ser Lys
             340             345             350
Pro Lys Gln Pro Pro Asp Ala Ser Pro Ala Asn Leu Leu Thr Ser Leu
             355             360             365
Val Arg Ala His Leu Asp Ser Gly Pro Ser Thr Ala Lys Leu Asp Tyr
             370             375             380
Ser Lys Phe Gln Glu Leu Val Leu Pro His Phe Gly Lys Glu Asp Ala
385             390             395             400
Gly Asp Val Gln Gln Phe Tyr Asp Leu Leu Ser Gly Ser Leu Glu Val
             405             410             415
Ile Arg Lys Trp Ala Glu Lys Ile Pro Gly Phe Ala Glu Leu Ser Pro
             420             425             430
Ala Asp Gln Asp Leu Leu Leu Glu Ser Ala Phe Leu Glu Leu Phe Ile
             435             440             445
Leu Arg Leu Ala Tyr Arg Ser Lys Pro Gly Glu Gly Lys Leu Ile Phe
             450             455             460
Cys Ser Gly Leu Val Leu His Arg Leu Gln Cys Ala Arg Gly Phe Gly
465             470             475             480
Asp Trp Ile Asp Ser Ile Leu Ala Phe Ser Arg Ser Leu His Ser Leu
             485             490             495
Leu Val Asp Val Pro Ala Phe Ala Cys Leu Ser Ala Leu Val Leu Ile
             500             505             510
Thr Asp Arg His Gly Leu Gln Glu Pro Arg Arg Val Glu Glu Leu Gln
             515             520             525
Asn Arg Ile Ala Ser Cys Leu Lys Glu His Val Ala Ala Val Ala Gly
             530             535             540
Glu Pro Gln Pro Ala Ser Cys Leu Ser Arg Leu Leu Gly Lys Leu Pro
545             550             555             560
Glu Leu Arg Thr Leu Cys Thr Gln Gly Leu Gln Arg Ile Phe Tyr Leu
             565             570             575
Lys Leu Glu Asp Leu Val Pro Pro Pro Ile Ile Asp Lys Ile Phe
             580             585             590
Met Asp Thr Leu Pro Phe
             595
```

<210> 7
<211> 1310
<212> DNA
<213> Homo Sapien

<400> 7
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tgggctcttc actcccaaca atggcggctc cgagcccgag cggcggcggc ggctccgggg   60
gcggcagcgg cagcggcacc cccggccccg tagggtcccc ggcgccaggc cacccggccg  120
tcagcagcat gcagggtaaa cgcaaagcac tgaagttgaa ttttgcaaat ccacctttca  180
aatctacagc aaggtttact ctgaatccca atcctacagg agttcaaaac ccacacatag  240
agagactgag aacacacagc attgagtcat caggaaaaact gaagatctcc cctgaacaac  300
actgggattt cactgcagag gacttgaaag accttggaga aattggacga ggagcttatg  360
gttctgtcaa caaaatggtc cacaaaccaa gtgggcaaat aatggcagtt aaaagaattc  420
```

```
ggtcaacagt ggatgaaaaa gaacaaaaac aacttcttat ggatttggat gtagtaatgc 480
ggagtagtga ttgcccatac attgttcagt tttatggtgc actcttcaga gagggtgact 540
gttggatctg tatggaactc atgtctacct cgtttgataa gttttacaaa tatgtatata 600
gtgtattaga tgatgttatt ccagaagaaa ttttaggcaa aatcacttta gcaactgtga 660
aagcactaaa ccacttaaaa gaaaacttga aaattattca cagagatatc aaaccttcca 720
atattcttct ggacagaagt ggaaatatta agctctgtga cttcggcatc agtggacagc 780
ttgtggactc tattgccaag acaagagatg ctggctgtag gccatacatg gcacctgaaa 840
gaatagaccc aagcgcatca cgacaaggat atgatgtccg ctctgatgtc tggagtttgg 900
ggatcacatt gtatgagttg ccacaggcc gatttcctta tccaaagtgg aatagtgtat 960
ttgatcaact aacacaagtc gtgaaaggag atcctccgca gctgagtaat tctgaggaaa 1020
gggaattctc cccgagtttc atcaactttg tcaacttgtg ccttacgaag gatgaatcca 1080
aaaggccaaa gtataaagag cttctgaaac atccctttat tttgatgtat gaagaacgtg 1140
ccgttgaggt cgcatgctat gtttgtaaaa tcctggatca aatgccagct actcccagct 1200
ctcccatgta tgtcgattga tatcgctgct acatcagact ctagaaaaaa gggctgagag 1260
gaagcaagac gtaaagaatt ttcatcccgt atcacagtgt ttttattgct           1310
```

```
<210> 8
<211> 399
<212> PRT
<213> Homo Sapien
```

```
<400> 8
Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Ser Gly Gly Gly Ser
1               5                   10                  15
Gly Ser Gly Thr Pro Gly Pro Val Gly Ser Pro Ala Pro Gly His Pro
            20                  25                  30
Ala Val Ser Ser Met Gln Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe
        35                  40                  45
Ala Asn Pro Pro Phe Lys Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn
    50                  55                  60
Pro Thr Gly Val Gln Asn Pro His Ile Glu Arg Leu Arg Thr His Ser
65                  70                  75                  80
Ile Glu Ser Ser Gly Lys Leu Lys Ile Ser Pro Glu Gln His Trp Asp
                85                  90                  95
Phe Thr Ala Glu Asp Leu Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala
            100                 105                 110
Tyr Gly Ser Val Asn Lys Met Val His Lys Pro Ser Gly Gln Ile Met
        115                 120                 125
Ala Val Lys Arg Ile Arg Ser Thr Val Asp Glu Lys Glu Gln Lys Gln
    130                 135                 140
Leu Leu Met Asp Leu Asp Val Val Met Arg Ser Ser Asp Cys Pro Tyr
145                 150                 155                 160
Ile Val Gln Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile
                165                 170                 175
Cys Met Glu Leu Met Ser Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val
            180                 185                 190
Tyr Ser Val Leu Asp Asp Val Ile Pro Glu Glu Ile Leu Gly Lys Ile
        195                 200                 205
Thr Leu Ala Thr Val Lys Ala Leu Asn His Leu Lys Glu Asn Leu Lys
    210                 215                 220
Ile Ile His Arg Asp Ile Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser
225                 230                 235                 240
Gly Asn Ile Lys Leu Cys Asp Phe Gly Ile Ser Gly Gln Leu Val Asp
                245                 250                 255
Ser Ile Ala Lys Thr Arg Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro
            260                 265                 270
Glu Arg Ile Asp Pro Ser Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser
        275                 280                 285
Asp Val Trp Ser Leu Gly Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg
    290                 295                 300
Phe Pro Tyr Pro Lys Trp Asn Ser Val Phe Asp Gln Leu Thr Gln Val
```

```
      305                     310                     315                     320
Val Lys Gly Asp Pro Pro Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe
                  325                     330                     335
Ser Pro Ser Phe Ile Asn Phe Val Asn Leu Cys Leu Thr Lys Asp Glu
              340                     345                     350
Ser Lys Arg Pro Lys Tyr Lys Glu Leu Leu Lys His Pro Phe Ile Leu
          355                     360                     365
Met Tyr Glu Glu Arg Ala Val Glu Val Ala Cys Tyr Val Cys Lys Ile
      370                     375                     380
Leu Asp Gln Met Pro Ala Thr Pro Ser Ser Pro Met Tyr Val Asp
385                     390                     395
```

<210> 9
<211> 1113
<212> DNA
<213> Homo Sapien

<400> 9

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atggctttgg aacagaacca gtcaacagat tattattatg aggaaaatga aatgaatggc 60
acttatgact acagtcaata tgaactgatc tgtatcaaag aagatgtcag agaatttgca 120
aaagttttcc tccctgtatt ccctcacaata gttttcgtca ttggacttgc aggcaattcc 180
atggtagtgg caatttatgc ctattacaag aaacagagaa ccaaaacaga tgtgtacatc 240
ctgaatttgg ctgtagcaga tttactcctt ctattcactc tgccttttg ggctgttaat 300
gcagttcatg ggtgggtttt agggaaaata atgtgcaaaa taacttcagc cttgtacaca 360
ctaaactttg tctctggaat gcagtttctg cttgtatca gcatagacag atatgtggca 420
gtaactaaag tccccagcca atcaggagtg ggaaaaccat gctggatcat ctgtttctgt 480
gtctggatgg ctgccatctt gctgagcata ccccagctgg ttttttatac agtaaatgac 540
aatgctaggt gcattcccat tttcccccgc tacctaggaa catcaatgaa agcattgatt 600
caaatgctag agatctgcat tggatttgta gtacccttc ttattatggg ggtgtgctac 660
tttatcacag caaggacact catgaagatg ccaaacatta aaatatctcg acccctaaaa 720
gttctgctca cagtcgttat agttttcatt gtcactcaac tgccttataa cattgtcaag 780
ttctgccgag ccatagacat catctactcc ctgatcacca gctgcaacat gagcaaacgc 840
atggacatcg ccatccaagt cacagaaagc atcgcactct tcacagctg cctcaacccca 900
atcctttatg tttttatggg agcatctttc aaaaactacg ttatgaaagt ggccaagaaa 960
tatgggtcct ggagaagaca gagacaaagt gtggaggagt ttccttttga ttctgagggt 1020
cctacagagc caaccagtac ttttagcatt taaaggtaaa actgctctgc cttttgcttg 1080
gatacatatg aatgatgctt tccccctcaaa taa 1113
```

<210> 10
<211> 350
<212> PRT
<213> Homo Sapien

<400> 10

```
Met Ala Leu Glu Gln Asn Gln Ser Thr Asp Tyr Tyr Tyr Glu Glu Asn
1               5                   10                  15
Glu Met Asn Gly Thr Tyr Asp Tyr Ser Gln Tyr Glu Leu Ile Cys Ile
              20                  25                  30
Lys Glu Asp Val Arg Glu Phe Ala Lys Val Phe Leu Pro Val Phe Leu
          35                  40                  45
Thr Ile Val Phe Val Ile Gly Leu Ala Gly Asn Ser Met Val Val Ala
      50                  55                  60
Ile Tyr Ala Tyr Tyr Lys Lys Gln Arg Thr Lys Thr Asp Val Tyr Ile
65                  70                  75                  80
Leu Asn Leu Ala Val Ala Asp Leu Leu Leu Phe Thr Leu Pro Phe
              85                  90                  95
Trp Ala Val Asn Ala Val His Gly Trp Val Leu Gly Lys Ile Met Cys
              100                 105                 110
Lys Ile Thr Ser Ala Leu Tyr Thr Leu Asn Phe Val Ser Gly Met Gln
          115                 120                 125
```

```
Phe Leu Ala Cys Ile Ser Ile Asp Arg Tyr Val Ala Val Thr Lys Val
    130               135               140
Pro Ser Gln Ser Gly Val Gly Lys Pro Cys Trp Ile Ile Cys Phe Cys
145               150               155               160
Val Trp Met Ala Ala Ile Leu Leu Ser Ile Pro Gln Leu Val Phe Tyr
              165               170               175
Thr Val Asn Asp Asn Ala Arg Cys Ile Pro Ile Phe Pro Arg Tyr Leu
        180               185               190
Gly Thr Ser Met Lys Ala Leu Ile Gln Met Leu Glu Ile Cys Ile Gly
        195               200               205
Phe Val Val Pro Phe Leu Ile Met Gly Val Cys Tyr Phe Ile Thr Ala
    210               215               220
Arg Thr Leu Met Lys Met Pro Asn Ile Lys Ile Ser Arg Pro Leu Lys
225               230               235               240
Val Leu Leu Thr Val Val Ile Val Phe Ile Val Thr Gln Leu Pro Tyr
              245               250               255
Asn Ile Val Lys Phe Cys Arg Ala Ile Asp Ile Ile Tyr Ser Leu Ile
              260               265               270
Thr Ser Cys Asn Met Ser Lys Arg Met Asp Ile Ala Ile Gln Val Thr
        275               280               285
Glu Ser Ile Ala Leu Phe His Ser Cys Leu Asn Pro Ile Leu Tyr Val
    290               295               300
Phe Met Gly Ala Ser Phe Lys Asn Tyr Val Met Lys Val Ala Lys Lys
305               310               315               320
Tyr Gly Ser Trp Arg Arg Gln Arg Gln Ser Val Glu Glu Phe Pro Phe
              325               330               335
Asp Ser Glu Gly Pro Thr Glu Pro Thr Ser Thr Phe Ser Ile
              340               345               350
```

<210> 11
<211> 1697
<212> DNA
<213> Homo Sapien

<400> 11

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actcccaaag tgctgggctt acaggtgtaa gccatcatgt ccagccgttc agatattcta 60
gttgaattgg agttggtggg ctagtacacc ttctaaatta aatgagtaaa ggatttagaa 120
tggtgcctga cacacagtag gtgctacatt catgttagct actattataa accttttcctg 180
cctctgactt tcagggtctt gcccaccacc agcgatgccc agcccttggt agagcttgaa 240
ccaccttcta taaacaggat ggcggtggag agacaggccc agtccctgag cccatgagga 300
gtgtggcccc ttcaggccca aagatgggga acatcactgc agacaactcc tcgatgagct 360
gtaccatcga ccataccatc caccagacgc tggccccggt ggtctatgtt accgtgctgg 420
tggtgggctt cccggccaac tgcctgtccc tctacttcgg ctacctgcag atcaaggccc 480
ggaacgagct gggcgtgtac ctgtgcaacc tgacggtggc cgacctcttc tacatctgct 540
cgctgccctt ctggctgcag tacgtgctgc agcacgacaa ctggtctcac ggcgacctgt 600
cctgccaggt gtgcggcatc ctcctgtacg agaacatcta tcagcgtg ggcttcctct 660
gctgcatctc cgtggaccgc tacctggctg tggcccatcc cttccgcttc accagttcc 720
ggaccctgaa ggcggccgtc ggcgtcagcg tggtcatctg ggccaaggag ctgctgacca 780
gcatctactt cctgatgcac gaggaggtca tcgaggacga gaaccagcac cgcgtgtgct 840
ttgagcacta ccccatccag gcatggcagc gcgccatcaa ctactaccgc ttcctggtgg 900
gcttcctctt ccccatctgc ctgctgctgg cgtcctacca gggcatcctg cgcgccgtgc 960
gccggagcca cggcacccag aagagccgca aggaccagat ccagcggctg gtgctcagca 1020
ccgtggtcat cttcctggcc tgcttcctgc cctaccacgt gttgctgctg gtgcgcagcg 1080
tctgggaggc cagctgcgac ttcgccaagg gcgtttcaa cgcctaccac ttctccctcc 1140
tgctcaccag cttcaactgc gtcgccgacc ccgtgctcta ctgcttcgtc agcgagacca 1200
cccaccggga cctggcccgc ctccgcgggg cctgctggc cttcctcacc tgctccagga 1260
ccggccgggc cagggaggcc tacccgctgg tgcccccga ggcctccggg aaaagcgggg 1320
cccagggtga ggagcccgag ctgttgacca agctccaccc cggccttcag acccctaact 1380
cgccagggtc gggcgggttc cccacgggca ggttggccta gctgggtcc tccgcggggtg 1440
gctccacgtg aggcctgagc cttcagccca cgggcctcag ggcctgccgc ctcctgcttc 1500
```

9

```
cctcgctgcg gaggcaggga agcccctgta actccggaag cctgctctcg cttgctgagc 1560
ccgctgggac cgccgagggt gggaataagc cccggttggc tcgtgggaat aagccgtgtc 1620
ctctgccgcg gctgcgatgt ggccacgctg gggctgctgg tcgggggaaa acagtgaact 1680
gcgtcccctg gcctgct                                                  1697
```

<210> 12
<211> 365
<212> PRT
<213> Homo Sapien

<400> 12

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Met Gly Asn Ile Thr Ala Asp Asn Ser Ser Met Ser Cys Thr Ile Asp
 1               5                  10                  15
His Thr Ile His Gln Thr Leu Ala Pro Val Val Tyr Val Thr Val Leu
            20                  25                  30
Val Val Gly Phe Pro Ala Asn Cys Leu Ser Leu Tyr Phe Gly Tyr Leu
        35                  40                  45
Gln Ile Lys Ala Arg Asn Glu Leu Gly Val Tyr Leu Cys Asn Leu Thr
    50                  55                  60
Val Ala Asp Leu Phe Tyr Ile Cys Ser Leu Pro Phe Trp Leu Gln Tyr
65                  70                  75                  80
Val Leu Gln His Asp Asn Trp Ser His Gly Asp Leu Ser Cys Gln Val
                85                  90                  95
Cys Gly Ile Leu Leu Tyr Glu Asn Ile Tyr Ile Ser Val Gly Phe Leu
            100                 105                 110
Cys Cys Ile Ser Val Asp Arg Tyr Leu Ala Val Ala His Pro Phe Arg
            115                 120                 125
Phe His Gln Phe Arg Thr Leu Lys Ala Ala Val Gly Val Ser Val Val
    130                 135                 140
Ile Trp Ala Lys Glu Leu Leu Thr Ser Ile Tyr Phe Leu Met His Glu
145                 150                 155                 160
Glu Val Ile Glu Asp Glu Asn Gln His Arg Val Cys Phe Glu His Tyr
                165                 170                 175
Pro Ile Gln Ala Trp Gln Arg Ala Ile Asn Tyr Tyr Arg Phe Leu Val
            180                 185                 190
Gly Phe Leu Phe Pro Ile Cys Leu Leu Leu Ala Ser Tyr Gln Gly Ile
            195                 200                 205
Leu Arg Ala Val Arg Arg Ser His Gly Thr Gln Lys Ser Arg Lys Asp
    210                 215                 220
Gln Ile Gln Arg Leu Val Leu Ser Thr Val Val Ile Phe Leu Ala Cys
225                 230                 235                 240
Phe Leu Pro Tyr His Val Leu Leu Leu Val Arg Ser Val Trp Glu Ala
                245                 250                 255
Ser Cys Asp Phe Ala Lys Gly Val Phe Asn Ala Tyr His Phe Ser Leu
            260                 265                 270
Leu Leu Thr Ser Phe Asn Cys Val Ala Asp Pro Val Leu Tyr Cys Phe
    275                 280                 285
Val Ser Glu Thr Thr His Arg Asp Leu Ala Arg Leu Arg Gly Ala Cys
    290                 295                 300
Leu Ala Phe Leu Thr Cys Ser Arg Thr Gly Arg Ala Arg Glu Ala Tyr
305                 310                 315                 320
Pro Leu Gly Ala Pro Glu Ala Ser Gly Lys Ser Gly Ala Gln Gly Glu
                325                 330                 335
Glu Pro Glu Leu Leu Thr Lys Leu His Pro Ala Phe Gln Thr Pro Asn
            340                 345                 350
Ser Pro Gly Ser Gly Gly Phe Pro Thr Gly Arg Leu Ala
    355                 360                 365
```


<210> 13
<211> 1181

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<212> DNA
<213> Homo Sapien

<400> 13
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tctgagaata gcagcttcta ttactatgac tacctggatg aagtggcctt catgctctgc 120
aggaaggatg cagtggtgtc ctttggcaaa gtcttcctcc cagtcttcta tagcctgatt 180
tttgtgttgg gcctcagcgg gaacctcctt cttctcatgg tcttgctccg ttacgtgcct 240
cgcaggcgga tggttgagat ctatctgctg aatctggcca tctccaacct tctgtttctg 300
gtgacactgc ccttctgggg catctccgtg gcctggcatt gggtcttcgg gagtttcttg 360
tgcaagatgg tgagcactct ttatactatt aacttttaca gtggcatctt tttcattagc 420
tgcatgagcc tggacaagta cctggagatc gttcatgctc agccctacca caggctgagg 480
acccgggcca gagcctgctc ccttgctacc atagtatggg ctgtgtccct ggccgtctcc 540
atccctgata tggtctttgt acagacacat gaaaatccca agggtgtgtg gaactgccac 600
gcagatttcg gcgggcatgg gaccatttgg aagctcttcc tccgcttcca gcagaacctc 660
ctagggtttc tccttccact ccttgccatg atcttcttct actcccgtat tggttgtgtc 720
ttggtgaggc tgaggcccgc aggccagggc cgggctttaa aaatagctgc agccttggtg 780
gtggccttct tcgtgctatg gttcccatac aatctcacct tgtttctgca tacgctgttg 840
gacctgcaag tattcgggaa ctgtgaggtc agccagcatc tagactacgc actccaggta 900
acagagagca tcgccttcct tcactgctgc ttttcccca tcctgtatgc cttctccagt 960
caccgcttcc gccagtacct gaaggctttc ctggctgccg tgcttggatg gcacctggca 1020
cctggcactg cccaggcctc attatccagc tgttctgaga gcagcatact tactgcccaa 1080
gaggaaatga ctggcatgaa tgaccttgga gagaggcagt ctgagaacta ccctaacaag 1140
gaggatgtgg ggaataaatc agcctgagtg accgcggccg c 1181

<210> 14
<211> 384
<212> PRT
<213> Homo Sapien

<400> 14
Met Ala Ala Thr Ala Ser Pro Gln Pro Leu Ala Thr Glu Asp Ala Asp
1               5                   10                  15
Ser Glu Asn Ser Ser Phe Tyr Tyr Tyr Asp Tyr Leu Asp Glu Val Ala
                20                  25                  30
Phe Met Leu Cys Arg Lys Asp Ala Val Val Ser Phe Gly Lys Val Phe
            35                  40                  45
Leu Pro Val Phe Tyr Ser Leu Ile Phe Val Leu Gly Leu Ser Gly Asn
        50                  55                  60
Leu Leu Leu Leu Met Val Leu Leu Arg Tyr Val Pro Arg Arg Arg Met
65                  70                  75                  80
Val Glu Ile Tyr Leu Leu Asn Leu Ala Ile Ser Asn Leu Leu Phe Leu
                85                  90                  95
Val Thr Leu Pro Phe Trp Gly Ile Ser Val Ala Trp His Trp Val Phe
            100                 105                 110
Gly Ser Phe Leu Cys Lys Met Val Ser Thr Leu Tyr Thr Ile Asn Phe
        115                 120                 125
Tyr Ser Gly Ile Phe Phe Ile Ser Cys Met Ser Leu Asp Lys Tyr Leu
    130                 135                 140
Glu Ile Val His Ala Gln Pro Tyr His Arg Leu Arg Thr Arg Ala Lys
145                 150                 155                 160
Ser Leu Leu Leu Ala Thr Ile Val Trp Ala Val Ser Leu Ala Val Ser
                165                 170                 175
Ile Pro Asp Met Val Phe Val Gln Thr His Glu Asn Pro Lys Gly Val
            180                 185                 190
Trp Asn Cys His Ala Asp Phe Gly Gly His Gly Thr Ile Trp Lys Leu
        195                 200                 205
Phe Leu Arg Phe Gln Gln Asn Leu Leu Gly Phe Leu Leu Pro Leu Leu
    210                 215                 220
Ala Met Ile Phe Phe Tyr Ser Arg Ile Gly Cys Val Leu Val Arg Leu
225                 230                 235                 240
```

11

Arg Pro Ala Gly Gln Gly Arg Ala Leu Lys Ile Ala Ala Ala Leu Val
                245   250   255
Val Ala Phe Phe Val Leu Trp Phe Pro Tyr Asn Leu Thr Leu Phe Leu
            260   265   270
His Thr Leu Leu Asp Leu Gln Val Phe Gly Asn Cys Glu Val Ser Gln
        275   280   285
His Leu Asp Tyr Ala Leu Gln Val Thr Glu Ser Ile Ala Phe Leu His
    290   295   300
Cys Cys Phe Ser Pro Ile Leu Tyr Ala Phe Ser Ser His Arg Phe Arg
305   310   315   320
Gln Tyr Leu Lys Ala Phe Leu Ala Ala Val Leu Gly Trp His Leu Ala
            325   330   335
Pro Gly Thr Ala Gln Ala Ser Leu Ser Ser Cys Ser Glu Ser Ser Ile
            340   345   350
Leu Thr Ala Gln Glu Glu Met Thr Gly Met Asn Asp Leu Gly Glu Arg
            355   360   365
Gln Ser Glu Asn Tyr Pro Asn Lys Glu Asp Val Gly Asn Lys Ser Ala
    370   375   380

<210> 15
<211> 1580
<212> DNA
<213> Homo Sapien

<400> 15
aaagaaggca tccagcaaga actgcacaag aaacggagtc agccggagaa caaggagtgg 60
tcttccactg cctcacagga ggatggaggc ccacaacgcg tctgccccat tcaacttcac 120
cctgccaccc aactttggca agcgccccac agacctggca ctgagcgtca tcctggtgtt 180
catgttgttc ttcatcatgc tctcgctggg ctgcaccatg gagttcagca agatcaaggc 240
tcacttatgg aagcctaaag ggctggccat cgccctggtg gcacagtatg gcatcatgcc 300
cctcacggcc tttgtgctgg gcaaggtctt ccggctgaag aacattgagg cactggccat 360
cttggtctgt ggctgctcac ctggagggaa cctgtccaat gtcttcagtc tggccatgaa 420
gggggacatg aacctcagca ttgtgatgac cacctgctcc accttctgtg cccttggcat 480
gatgcctctc ctcctgtaca tctactccag ggggatctat gatggggacc tgaaggacaa 540
ggtgccctat aaaggcatcg tgatatcact ggtcctggtt ctcattcctt gcaccatagg 600
gatcgtcctc aaatccaaac ggccacaata catgcgctat gtcatcaagg agggatgat 660
catcattctc ttgtgcagtg tggccgtcac agttctctct gccatcaatg tggggaagag 720
catcatgttt gccatgacac cactcttgat tgccacctcc tccctgatgc cttttattgg 780
ctttctgctg ggttatgttc tctctgctct cttctgcctc aatggacggt gcagacgcac 840
tgtcagcatg gagactggat gccaaaatgt ccaactctgt tccaccatcc tcaatgtggc 900
ctttccacct gaagtcattg gaccactttt cttctttccc ctcctctaca tgatttttcca 960
gcttggagaa gggcttctcc tcattgccat attttggtgc tatgagaaat tcaagactcc 1020
caaggataaa acaaaaatga tctacacagc tgccacaact gaagaaacaa ttccaggagc 1080
tctgggaaat ggcacctaca aggggagga ctgctcccct tgcacagcct agcccttccc 1140
ctggtggcct ggattctggt cccaaagcaa ttctgaaagc cagtgtggta aactagagag 1200
agcagcaaaa acaccagtct tgcctgagtc tttctccagc atttccagta catctatcag 1260
aatcatcaag tcttggccgg aacacagac agggtgtcta cccaagaagc ctcacctatc 1320
cccaacttag aatttgctac ttattttaaa gacttgttca gtgactgtaa actctatgaa 1380
accagaaacc gaatctgcct cttgctggga tctctaaaag tgtctgataa gcatcttaaa 1440
gtcactcaat tcctgaacta atcaatatat atgtttaacc cattactcaa atacccaaat 1500
cccattccaa gtttgtgac ccaaaagaga aataaatgct cacaagtgct gtagaattaa 1560
acttcagaag ttctaacctt   1580

<210> 16
<211> 349
<212> PRT
<213> Homo Sapien

<400> 16
Met Glu Ala His Asn Ala Ser Ala Pro Phe Asn Phe Thr Leu Pro Pro

```
  1              5                   10                  15
Asn Phe Gly Lys Arg Pro Thr Asp Leu Ala Leu Ser Val Ile Leu Val
            20                  25                  30
Phe Met Leu Phe Phe Ile Met Leu Ser Leu Gly Cys Thr Met Glu Phe
        35                  40                  45
Ser Lys Ile Lys Ala His Leu Trp Lys Pro Lys Gly Leu Ala Ile Ala
        50                  55                  60
Leu Val Ala Gln Tyr Gly Ile Met Pro Leu Thr Ala Phe Val Leu Gly
65                  70                  75                  80
Lys Val Phe Arg Leu Lys Asn Ile Glu Ala Leu Ala Ile Leu Val Cys
                85                  90                  95
Gly Cys Ser Pro Gly Gly Asn Leu Ser Asn Val Phe Ser Leu Ala Met
            100                 105                 110
Lys Gly Asp Met Asn Leu Ser Ile Val Met Thr Thr Cys Ser Thr Phe
            115                 120                 125
Cys Ala Leu Gly Met Met Pro Leu Leu Leu Tyr Ile Tyr Ser Arg Gly
130                 135                 140
Ile Tyr Asp Gly Asp Leu Lys Asp Lys Val Pro Tyr Lys Gly Ile Val
145                 150                 155                 160
Ile Ser Leu Val Leu Val Leu Ile Pro Cys Thr Ile Gly Ile Val Leu
            165                 170                 175
Lys Ser Lys Arg Pro Gln Tyr Met Arg Tyr Val Ile Lys Gly Gly Met
            180                 185                 190
Ile Ile Ile Leu Leu Cys Ser Val Ala Val Thr Val Leu Ser Ala Ile
        195                 200                 205
Asn Val Gly Lys Ser Ile Met Phe Ala Met Thr Pro Leu Leu Ile Ala
    210                 215                 220
Thr Ser Ser Leu Met Pro Phe Ile Gly Phe Leu Leu Gly Tyr Val Leu
225                 230                 235                 240
Ser Ala Leu Phe Cys Leu Asn Gly Arg Cys Arg Arg Thr Val Ser Met
            245                 250                 255
Glu Thr Gly Cys Gln Asn Val Gln Leu Cys Ser Thr Ile Leu Asn Val
            260                 265                 270
Ala Phe Pro Pro Glu Val Ile Gly Pro Leu Phe Phe Phe Pro Leu Leu
        275                 280                 285
Tyr Met Ile Phe Gln Leu Gly Glu Gly Leu Leu Leu Ile Ala Ile Phe
    290                 295                 300
Trp Cys Tyr Glu Lys Phe Lys Thr Pro Lys Asp Lys Thr Lys Met Ile
305                 310                 315                 320
Tyr Thr Ala Ala Thr Thr Glu Glu Thr Ile Pro Gly Ala Leu Gly Asn
            325                 330                 335
Gly Thr Tyr Lys Gly Glu Asp Cys Ser Pro Cys Thr Ala
            340                 345
```

<210> 17
<211> 2013
<212> DNA
<213> Homo Sapien

<400> 17
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aattcctgtc ctcaggcgct gtcagatctg tggttttttct acttgaagga cacaatgttt 60
tccaaactag cacatttgca gaggtttgct gtacttagtc gcggagttca ttcttcagtg 120
gcttctgcta catctgttgc aactaaaaaa acagtccaag gccctccaac ctctgatgac 180
attttgaaa gggaatataa gtatggtgca cacaactacc atcctttacc tgtagccctg 240
gagagaggaa aaggtattta cttatgggat gtagaaggca gaaaatattt tgacttcctg 300
agttcttaca gtgctgtcaa ccaagggcat tgtcacccca agattgtgaa tgctctgaag 360
agtcaagtgg acaaattgac cttaacatct agagctttct ataataacgt acttggtgaa 420
tatgaggagt atattactaa acttttcaac taccacaaag ttcttcctat gaatacagga 480
gtggaggctg agagactgc ctgtaaacta gctcgtaagt ggggctatac cgtgaagggc 540
attcagaaat acaaagcaaa gattgttttt gcagctggga acttctgggg taggacgttg 600
```

```
tctgctatct ccagttccac agacccaacc agttacgatg gttttggacc atttatgccg 660
ggattcgaca tcattcccta taatgatctg cccgcactgg agcgtgctct tcaggatcca 720
aatgtggctg cgttcatggt agaaccaatt cagggtgaag caggcgttgt tgttccggat 780
ccaggttacc taatgggagt gcgagagctc tgcaccaggc accaggttct ctttattgct 840
gatgaaatac agacaggatt ggccagaact ggtagatggc tggctgttga ttatgaaaat 900
gtcagacctg atatagtcct ccttggaaag gcccttctg ggggcttata ccctgtgtct 960
gcagtgctgt gtgatgatga catcatgctg accattaagc aggggagca tgggtccaca 1020
tacggtggca atccactagg ctgccgagtg gccatcgcag cccttgaggt tttagaagaa 1080
gaaaaccttg ctgaaaatgc agacaaattg ggcattatct tgagaaatga actcatgaag 1140
ctaccttctg atgttgtaac tgccgtaaga ggaaaaggat tattaaacgc tattgtcatt 1200
aaagaaacca aagattggga tgcttggaag gtgtgtctac gacttcgaga taatggactt 1260
ctggccaagc caacccatgg cgacattatc aggtttgcgc ctccgctggt gatcaaggag 1320
gatgagcttc gagagtccat tgaaattatt aacaagacca tcttgtcttt ctgagggtag 1380
ccagctgttt tcagtggtcc ctgggagcca gctggagaca ggtggtcctg taaaagcttt 1440
attcctaatg tgggcacatt ccactcccat gagtcttcaa aaacttttt tttgaatata 1500
tttttttcag ttgatacata atagaacaac gtttatgaac ctgccgtttg ctttgtaacg 1560
taactaaata atgtaatggc atctatattc agttgaagtg ttttgatgtg catgtgtact 1620
tcctaaggtg aaatgcatct atatacagac agcctctaaa tcaagtcctt cagtataatt 1680
gatatatgtt tttataattt cctcactggt ataagtgttt catatttgaa aaagttatct 1740
ctgggtattg cataaaaggc ttcatcttat aaagtgaaat cattgttatt gaattttagg 1800
aaggattaat ggttaagtgt atataaaata ctaatattaa gtaaacttca tattggccaa 1860
caccagggtt gtattctatg gatgtcatta ttttgaatta agaattagtg tttaacattc 1920
ctaaattgtt ttgagtgctt gattataatt tgtaaaaaat gtttattttc aatacttctt 1980
taaatttaaa ataaagctta tatttcaaat gtc                              2013
```

```
<210> 18
<211> 439
<212> PRT
<213> Homo Sapien
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```
<400> 18
Met Phe Ser Lys Leu Ala His Leu Gln Arg Phe Ala Val Leu Ser Arg
  1               5                   10                  15
Gly Val His Ser Ser Val Ala Ser Ala Thr Ser Val Ala Thr Lys Lys
             20                  25                  30
Thr Val Gln Gly Pro Pro Thr Ser Asp Asp Ile Phe Glu Arg Glu Tyr
         35                  40                  45
Lys Tyr Gly Ala His Asn Tyr His Pro Leu Pro Val Ala Leu Glu Arg
     50                  55                  60
Gly Lys Gly Ile Tyr Leu Trp Asp Val Glu Gly Arg Lys Tyr Phe Asp
 65                  70                  75                  80
Phe Leu Ser Ser Tyr Ser Ala Val Asn Gln Gly His Cys His Pro Lys
                 85                  90                  95
Ile Val Asn Ala Leu Lys Ser Gln Val Asp Lys Leu Thr Leu Thr Ser
            100                 105                 110
Arg Ala Phe Tyr Asn Asn Val Leu Gly Glu Tyr Glu Glu Tyr Ile Thr
            115                 120                 125
Lys Leu Phe Asn Tyr His Lys Val Leu Pro Met Asn Thr Gly Val Glu
        130                 135                 140
Ala Gly Glu Thr Ala Cys Lys Leu Ala Arg Lys Trp Gly Tyr Thr Val
145                 150                 155                 160
Lys Gly Ile Gln Lys Tyr Lys Ala Lys Ile Val Phe Ala Ala Gly Asn
                165                 170                 175
Phe Trp Gly Arg Thr Leu Ser Ala Ile Ser Ser Ser Thr Asp Pro Thr
            180                 185                 190
Ser Tyr Asp Gly Phe Gly Pro Phe Met Pro Gly Phe Asp Ile Ile Pro
        195                 200                 205
Tyr Asn Asp Leu Pro Ala Leu Glu Arg Ala Leu Gln Asp Pro Asn Val
    210                 215                 220
Ala Ala Phe Met Val Glu Pro Ile Gln Gly Glu Ala Gly Val Val Val
225                 230                 235                 240
```

14

```
Pro Asp Pro Gly Tyr Leu Met Gly Val Arg Glu Leu Cys Thr Arg His
             245                 250                 255
Gln Val Leu Phe Ile Ala Asp Glu Ile Gln Thr Gly Leu Ala Arg Thr
             260                 265                 270
Gly Arg Trp Leu Ala Val Asp Tyr Glu Asn Val Arg Pro Asp Ile Val
         275                 280                 285
Leu Leu Gly Lys Ala Leu Ser Gly Gly Leu Tyr Pro Val Ser Ala Val
     290                 295                 300
Leu Cys Asp Asp Asp Ile Met Leu Thr Ile Lys Pro Gly Glu His Gly
305                 310                 315                 320
Ser Thr Tyr Gly Gly Asn Pro Leu Gly Cys Arg Val Ala Ile Ala Ala
             325                 330                 335
Leu Glu Val Leu Glu Glu Glu Asn Leu Ala Glu Asn Ala Asp Lys Leu
             340                 345                 350
Gly Ile Ile Leu Arg Asn Glu Leu Met Lys Leu Pro Ser Asp Val Val
         355                 360                 365
Thr Ala Val Arg Gly Lys Gly Leu Leu Asn Ala Ile Val Ile Lys Glu
     370                 375                 380
Thr Lys Asp Trp Asp Ala Trp Lys Val Cys Leu Arg Leu Arg Asp Asn
385                 390                 395                 400
Gly Leu Leu Ala Lys Pro Thr His Gly Asp Ile Ile Arg Phe Ala Pro
             405                 410                 415
Pro Leu Val Ile Lys Glu Asp Glu Leu Arg Glu Ser Ile Glu Ile Ile
             420                 425                 430
Asn Lys Thr Ile Leu Ser Phe
             435
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```
<210> 19
<211> 1227
<212> DNA
<213> Homo Sapien
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<400> 19
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cactggcaaa cagggcacct ttcacagcca gcaggcattg gaatatggca ccaaactcgt 240
tggaggaaca ctcccaggga aaggaggcca gacacatctg gcttacctg tctttaatac 300
tgtgaaggag gccaaagaac agacaggagc aacggcttct gtcattatg ttcctccgcc 360
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cactgaagga attccccagc aggacatggt acgagtcaag cacaaactgc tgcgccagga 480
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caccctgact tatgaagcag ttcaccaaac aacgcaagtt ggattggggc agtctttgtg 660
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taacccagca gcagtttgct tctgttgtcc actgattaat cagcctatgt gcctgacact 1140
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```

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<210> 20
<211> 333
<212> PRT
<213> Homo Sapien
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15

<400> 20
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            20                  25                  30
Ser Arg Gln His Leu Tyr Val Asp Lys Asn Thr Lys Ile Ile Cys Gln
        35                  40                  45
Gly Phe Thr Gly Lys Gln Gly Thr Phe His Ser Gln Gln Ala Leu Glu
    50                  55                  60
Tyr Gly Thr Lys Leu Val Gly Gly Thr Leu Pro Gly Lys Gly Gly Gln
65                  70                  75                  80
Thr His Leu Gly Leu Pro Val Phe Asn Thr Val Lys Glu Ala Lys Glu
            85                  90                  95
Gln Thr Gly Ala Thr Ala Ser Val Ile Tyr Val Pro Pro Pro Phe Ala
        100                 105                 110
Ala Ala Ala Ile Asn Glu Ala Ile Glu Ala Glu Ile Pro Leu Val Val
        115                 120                 125
Cys Ile Thr Glu Gly Ile Pro Gln Gln Asp Met Val Arg Val Lys His
    130                 135                 140
Lys Leu Leu Arg Gln Glu Lys Thr Arg Leu Ile Gly Pro Asn Cys Pro
145                 150                 155                 160
Gly Val Ile Asn Pro Gly Glu Cys Lys Ile Gly Ile Met Pro Gly His
                165                 170                 175
Ile His Lys Lys Gly Arg Ile Gly Ile Val Ser Arg Ser Gly Thr Leu
            180                 185                 190
Thr Tyr Glu Ala Val His Gln Thr Thr Gln Val Gly Leu Gly Gln Ser
        195                 200                 205
Leu Cys Val Gly Ile Gly Gly Asp Pro Phe Asn Gly Thr Asp Phe Ile
    210                 215                 220
Asp Cys Leu Glu Ile Phe Leu Asn Asp Ser Ala Thr Glu Gly Ile Ile
225                 230                 235                 240
Leu Ile Gly Glu Ile Gly Gly Asn Ala Glu Glu Asn Ala Ala Glu Phe
                245                 250                 255
Leu Lys Gln His Asn Ser Gly Pro Asn Ser Lys Pro Val Val Ser Phe
            260                 265                 270
Ile Ala Gly Leu Thr Ala Pro Pro Gly Arg Arg Met Gly His Ala Gly
        275                 280                 285
Ala Ile Ile Ala Gly Gly Lys Gly Gly Ala Lys Glu Lys Ile Ser Ala
        290                 295                 300
Leu Gln Ser Ala Gly Val Val Val Ser Met Ser Pro Ala Gln Leu Gly
305                 310                 315                 320
Thr Thr Ile Tyr Lys Glu Phe Glu Lys Arg Lys Met Leu
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```

<210> 21
<211> 4040
<212> DNA
<213> Homo Sapien

<400> 21
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gcgacacctc tactagccga gccggccgct gtgcccgctc ggtcttcggc aacattaagg 180
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tttccagctt gaatgagatc agcaatgcca tcctcatcat ctttgtcagc tactttggca 360
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tcatcctcac cctcccacac ttcctctccg agccctacca gtacaccttg ccagcactg 480
ggaacaacag ccgcttgcag gccgagctct gccagaagca ttggcaggac ctgcctccca 540
gtaagtgcca cagcaccacc cagaaccccc agaaggagac cagcagcatg tggggcctga 600
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ccatctctgt atttggaccg gctttcgggt acctgctggg ctctatcatg ctgcagatct 780
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<210> 22
<211> 643

17

<212> PRT
<213> Homo Sapien

<400> 22

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Thr Ser Arg Ala Gly Arg Cys Ala Arg Ser Val Phe Gly Asn Ile Lys
            20                  25                  30
Val Phe Val Leu Cys Gln Gly Leu Leu Gln Leu Cys Gln Leu Leu Tyr
        35                  40                  45
Ser Ala Tyr Phe Lys Ser Ser Leu Thr Thr Ile Glu Lys Arg Phe Gly
    50                  55                  60
Leu Ser Ser Ser Ser Ser Gly Leu Ile Ser Ser Leu Asn Glu Ile Ser
65                  70                  75                  80
Asn Ala Ile Leu Ile Ile Phe Val Ser Tyr Phe Gly Ser Arg Val His
                85                  90                  95
Arg Pro Arg Leu Ile Gly Ile Gly Gly Leu Phe Leu Ala Ala Gly Ala
            100                 105                 110
Phe Ile Leu Thr Leu Pro His Phe Leu Ser Glu Pro Tyr Gln Tyr Thr
        115                 120                 125
Leu Ala Ser Thr Gly Asn Asn Ser Arg Leu Gln Ala Glu Leu Cys Gln
    130                 135                 140
Lys His Trp Gln Asp Leu Pro Pro Ser Lys Cys His Ser Thr Thr Gln
145                 150                 155                 160
Asn Pro Gln Lys Glu Thr Ser Ser Met Trp Gly Leu Met Val Val Ala
                165                 170                 175
Gln Leu Leu Ala Gly Ile Gly Thr Val Pro Ile Gln Pro Phe Gly Ile
            180                 185                 190
Ser Tyr Val Asp Asp Phe Ser Glu Pro Ser Asn Ser Pro Leu Tyr Ile
        195                 200                 205
Ser Ile Leu Phe Ala Ile Ser Val Phe Gly Pro Ala Phe Gly Tyr Leu
    210                 215                 220
Leu Gly Ser Ile Met Leu Gln Ile Phe Val Asp Tyr Gly Arg Val Asn
225                 230                 235                 240
Thr Ala Ala Val Asn Leu Val Pro Gly Asp Pro Arg Trp Ile Gly Ala
                245                 250                 255
Trp Trp Leu Gly Leu Leu Ile Ser Ser Ala Leu Leu Val Leu Thr Ser
            260                 265                 270
Phe Pro Phe Phe Phe Phe Pro Arg Ala Met Pro Ile Gly Ala Lys Arg
        275                 280                 285
Ala Pro Ala Thr Ala Asp Glu Ala Arg Lys Leu Glu Glu Ala Lys Ser
    290                 295                 300
Arg Gly Ser Leu Val Asp Phe Ile Lys Arg Phe Pro Cys Ile Phe Leu
305                 310                 315                 320
Arg Leu Leu Met Asn Ser Leu Phe Val Leu Val Val Leu Ala Gln Cys
                325                 330                 335
Thr Phe Ser Ser Val Ile Ala Gly Leu Ser Thr Phe Leu Asn Lys Phe
            340                 345                 350
Leu Glu Lys Gln Tyr Gly Thr Ser Ala Ala Tyr Ala Asn Phe Leu Ile
        355                 360                 365
Gly Ala Val Asn Leu Pro Ala Ala Ala Leu Gly Met Leu Phe Gly Gly
    370                 375                 380
Ile Leu Met Lys Arg Phe Val Phe Ser Leu Gln Thr Ile Pro Arg Ile
385                 390                 395                 400
Ala Thr Thr Ile Ile Thr Ile Ser Met Ile Leu Cys Val Pro Leu Phe
                405                 410                 415
Phe Met Gly Cys Ser Thr Pro Thr Val Ala Glu Val Tyr Pro Pro Ser
            420                 425                 430
Thr Ser Ser Ser Ile His Pro Gln Ser Pro Ala Cys Arg Arg Asp Cys
        435                 440                 445
Ser Cys Pro Asp Ser Ile Phe His Pro Val Cys Gly Asp Asn Gly Ile
```

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      450                      455                      460
Glu Tyr Leu Ser Pro Cys His Ala Gly Cys Ser Asn Ile Asn Met Ser
465                      470                      475                      480
Ser Ala Thr Ser Lys Gln Leu Ile Tyr Leu Asn Cys Ser Cys Val Thr
                485                      490                      495
Gly Gly Ser Ala Ser Ala Lys Thr Gly Ser Cys Pro Val Pro Cys Ala
            500                      505                      510
His Phe Leu Leu Pro Ala Ile Phe Leu Ile Ser Phe Val Ser Leu Ile
            515                      520                      525
Ala Cys Ile Ser His Asn Pro Leu Tyr Met Met Val Leu Arg Val Val
        530                      535                      540
Asn Gln Glu Glu Lys Ser Phe Ala Ile Gly Val Gln Phe Leu Leu Met
545                      550                      555                      560
Arg Leu Leu Ala Trp Leu Pro Ser Pro Ala Leu Tyr Gly Leu Thr Ile
                565                      570                      575
Asp His Ser Cys Ile Arg Trp Asn Ser Leu Cys Leu Gly Arg Arg Gly
        580                      585                      590
Ala Cys Ala Tyr Tyr Asp Asn Asp Ala Leu Arg Asp Arg Tyr Leu Gly
        595                      600                      605
Leu Gln Met Gly Tyr Lys Ala Leu Gly Met Leu Leu Leu Cys Phe Ile
        610                      615                      620
Ser Trp Arg Val Lys Lys Asn Lys Glu Tyr Asn Val Gln Lys Ala Ala
625                      630                      635                      640
Gly Leu Ile
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<210> 23
<211> 4897
<212> DNA
<213> Homo Sapien

<400> 23

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19

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<211> 506
<212> PRT
<213> Homo Sapien

<400> 24

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Ser Ser Ser Tyr Ser Ser Asn Ser Asp Phe Asn Tyr Ser Tyr Pro Thr
            20                  25                  30
Lys Gln Ala Ala Leu Lys Ser His Tyr Ala Asp Val Asp Pro Glu Asn
        35                  40                  45
Gln Asn Phe Leu Leu Glu Ser Asn Leu Gly Lys Lys Lys Tyr Glu Thr
    50                  55                  60
Glu Phe His Pro Gly Thr Thr Ser Phe Gly Met Ser Val Phe Asn Leu
65              70                  75                  80
Ser Asn Ala Ile Val Gly Ser Gly Ile Leu Gly Leu Ser Tyr Ala Met
                85                  90                  95
Ala Asn Thr Gly Ile Ala Leu Phe Ile Ile Leu Leu Thr Phe Val Ser
            100                 105                 110
Ile Phe Ser Leu Tyr Ser Val His Leu Leu Leu Lys Thr Ala Asn Glu
        115                 120                 125
Gly Gly Ser Leu Leu Tyr Glu Gln Leu Gly Tyr Lys Ala Phe Gly Leu
    130                 135                 140
Val Gly Lys Leu Ala Ala Ser Gly Ser Ile Thr Met Gln Asn Ile Gly
145                 150                 155                 160
Ala Met Ser Ser Tyr Leu Phe Ile Val Lys Tyr Glu Leu Pro Leu Val
                165                 170                 175
Ile Gln Ala Leu Thr Asn Ile Glu Asp Lys Thr Gly Leu Trp Tyr Leu
        180                 185                 190
Asn Gly Asn Tyr Leu Val Leu Leu Val Ser Leu Val Val Ile Leu Pro
    195                 200                 205
Leu Ser Leu Phe Arg Asn Leu Gly Tyr Leu Gly Tyr Thr Ser Gly Leu
    210                 215                 220
Ser Leu Leu Cys Met Val Phe Phe Leu Ile Val Val Ile Cys Lys Lys
225                 230                 235                 240
Phe Gln Val Pro Cys Pro Val Glu Ala Ala Leu Ile Ile Asn Glu Thr
                245                 250                 255
Ile Asn Thr Thr Leu Thr Gln Pro Thr Ala Leu Val Pro Ala Leu Ser
        260                 265                 270
His Asn Val Thr Glu Asn Asp Ser Cys Arg Pro His Tyr Phe Ile Phe
        275                 280                 285
Asn Ser Gln Thr Val Tyr Ala Val Pro Ile Leu Ile Phe Ser Phe Val
290                 295                 300
Cys His Pro Ala Val Leu Pro Ile Tyr Glu Glu Leu Lys Asp Arg Ser
305                 310                 315                 320
Arg Arg Arg Met Met Asn Val Ser Lys Ile Ser Phe Phe Ala Met Phe
                325                 330                 335
Leu Met Tyr Leu Leu Ala Ala Leu Phe Gly Tyr Leu Thr Phe Tyr Glu
        340                 345                 350
His Val Glu Ser Glu Leu Leu His Thr Tyr Ser Ser Ile Leu Gly Thr
        355                 360                 365
Asp Ile Leu Leu Leu Ile Val Arg Leu Ala Val Leu Met Ala Val Thr
370                 375                 380
Leu Thr Val Pro Val Val Ile Phe Pro Ile Arg Ser Ser Val Thr His
385                 390                 395                 400
Leu Leu Cys Ala Ser Lys Asp Phe Ser Trp Trp Arg His Ser Leu Ile
            405                 410                 415
Thr Val Ser Ile Leu Ala Phe Thr Asn Leu Leu Val Ile Phe Val Pro
        420                 425                 430
Thr Ile Arg Asp Ile Phe Gly Phe Ile Gly Ala Ser Ala Ala Ser Met
        435                 440                 445
Leu Ile Phe Ile Leu Pro Ser Ala Phe Tyr Ile Lys Leu Val Lys Lys
    450                 455                 460
Glu Pro Met Lys Ser Val Gln Lys Ile Gly Ala Leu Phe Phe Leu Leu
465                 470                 475                 480
Ser Gly Val Leu Val Met Thr Gly Ser Met Ala Leu Ile Val Leu Asp
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                     485                    490                    495
Trp Val His Asn Ala Pro Gly Gly Gly His
             500                    505


<210> 25
<211> 2106
<212> DNA
<213> Homo Sapien

<400> 25
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gtcccctcgc tgcagcgcta ccgcgagctg caccggcgct ccgtggagga gccgcgggaa 180
ttctggggga cacattgcaa ggaattttac tggaagactc catgccctgg cccattcctt 240
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accaacatct gctacaatgt actggatcga aatgtccatg agaaaaagct tggagataaa 360
gttgctttt actgggaggg caatgagcca ggggagacca ctcagatcac ataccatcag 420
cttctggtcc aagtgtgtca gttcagcaat gttctccgaa aacagggcat tcagaagggg 480
gaccgagtgg ccatctacat gcctatgatc ccagagcttg tggtggccat gctggcatgt 540
gcccgcattg gggctttgca ctccattgtg tttgcaggct ctcttcaga gtctctatgt 600
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tattactgga tcactggcag gattgatgac atgctcaatg tatctggaca cctgctgagt 1740
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aaaatcatga ggcgagtgct tcggaagatt gctcagaatg accatgacct cggggacatg 2040
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cagtga                                                          2106


<210> 26
<211> 701
<212> PRT
<213> Homo Sapien

<400> 26
Met Gly Leu Pro Glu Glu Arg Val Arg Ser Gly Ser Gly Ser Arg Gly
1               5                   10                  15
Gln Glu Glu Ala Gly Ala Gly Gly Arg Ala Arg Ser Trp Ser Pro Pro
            20                  25                  30
Pro Glu Val Ser Arg Ser Ala His Val Pro Ser Leu Gln Arg Tyr Arg
        35                  40                  45
Glu Leu His Arg Arg Ser Val Glu Glu Pro Arg Glu Phe Trp Gly Asp
```

```
          50                    55                    60
Ile Ala Lys Glu Phe Tyr Trp Lys Thr Pro Cys Pro Gly Pro Phe Leu
65                    70                    75                    80
Arg Tyr Asn Phe Asp Val Thr Lys Gly Lys Ile Phe Ile Glu Trp Met
               85                    90                    95
Lys Gly Ala Thr Thr Asn Ile Cys Tyr Asn Val Leu Asp Arg Asn Val
               100                   105                   110
His Glu Lys Lys Leu Gly Asp Lys Val Ala Phe Tyr Trp Glu Gly Asn
          115                   120                   125
Glu Pro Gly Glu Thr Thr Gln Ile Thr Tyr His Gln Leu Leu Val Gln
     130                   135                   140
Val Cys Gln Phe Ser Asn Val Leu Arg Lys Gln Gly Ile Gln Lys Gly
145                   150                   155                   160
Asp Arg Val Ala Ile Tyr Met Pro Met Ile Pro Glu Leu Val Val Ala
               165                   170                   175
Met Leu Ala Cys Ala Arg Ile Gly Ala Leu His Ser Ile Val Phe Ala
               180                   185                   190
Gly Phe Ser Ser Glu Ser Leu Cys Glu Arg Ile Leu Asp Ser Ser Cys
          195                   200                   205
Ser Leu Leu Ile Thr Thr Asp Ala Phe Tyr Arg Gly Glu Lys Leu Val
     210                   215                   220
Asn Leu Lys Glu Leu Ala Asp Glu Ala Leu Gln Lys Cys Gln Glu Lys
225                   230                   235                   240
Gly Phe Pro Val Arg Cys Cys Ile Val Val Lys His Leu Gly Arg Ala
               245                   250                   255
Glu Leu Gly Met Gly Asp Ser Thr Ser Gln Ser Pro Pro Ile Lys Arg
               260                   265                   270
Ser Cys Pro Asp Val Gln Ile Ser Trp Asn Gln Gly Ile Asp Leu Trp
          275                   280                   285
Trp His Glu Leu Met Gln Glu Ala Gly Asp Glu Cys Glu Pro Glu Trp
     290                   295                   300
Cys Asp Ala Glu Asp Pro Leu Phe Ile Leu Tyr Thr Ser Gly Ser Thr
305                   310                   315                   320
Gly Lys Pro Lys Gly Val Val His Thr Val Gly Gly Tyr Met Leu Tyr
               325                   330                   335
Val Ala Thr Thr Phe Lys Tyr Val Phe Asp Phe His Ala Glu Asp Val
               340                   345                   350
Phe Trp Cys Thr Ala Asp Ile Gly Trp Ile Thr Gly His Ser Tyr Val
          355                   360                   365
Thr Tyr Gly Pro Leu Ala Asn Gly Ala Thr Ser Val Leu Phe Glu Gly
     370                   375                   380
Ile Pro Thr Tyr Pro Asp Val Asn Arg Leu Trp Ser Ile Val Asp Lys
385                   390                   395                   400
Tyr Lys Val Thr Lys Phe Tyr Thr Ala Pro Thr Ala Ile Arg Leu Leu
               405                   410                   415
Met Lys Phe Gly Asp Glu Pro Val Thr Lys His Ser Arg Ala Ser Leu
          420                   425                   430
Gln Val Leu Gly Thr Val Gly Glu Pro Ile Asn Pro Glu Ala Trp Leu
     435                   440                   445
Trp Tyr His Arg Val Val Gly Ala Gln Arg Cys Pro Ile Val Asp Thr
     450                   455                   460
Phe Trp Gln Thr Glu Thr Gly Gly His Met Leu Thr Pro Leu Pro Gly
465                   470                   475                   480
Ala Thr Pro Met Lys Pro Gly Ser Ala Thr Phe Pro Phe Phe Gly Val
               485                   490                   495
Ala Pro Ala Ile Leu Asn Glu Ser Gly Glu Glu Leu Glu Gly Glu Ala
          500                   505                   510
Glu Gly Tyr Leu Val Phe Lys Gln Pro Trp Pro Gly Ile Met Arg Thr
          515                   520                   525
Val Tyr Gly Asn His Glu Arg Phe Glu Thr Thr Tyr Phe Lys Lys Phe
     530                   535                   540
```

```
Pro Gly Tyr Tyr Val Thr Gly Asp Gly Cys Gln Arg Asp Gln Asp Gly
545             550             555             560
Tyr Tyr Trp Ile Thr Gly Arg Ile Asp Asp Met Leu Asn Val Ser Gly
            565             570             575
His Leu Leu Ser Thr Ala Glu Val Glu Ser Ala Leu Val Glu His Glu
        580             585             590
Ala Val Ala Glu Ala Ala Val Val Gly His Pro His Pro Val Lys Gly
    595             600             605
Glu Cys Leu Tyr Cys Phe Val Thr Leu Cys Asp Gly His Thr Phe Ser
    610             615             620
Pro Lys Leu Thr Glu Glu Leu Lys Lys Gln Ile Arg Glu Lys Ile Gly
625             630             635             640
Pro Ile Ala Thr Pro Asp Tyr Ile Gln Asn Ala Pro Gly Leu Pro Lys
                645             650             655
Thr Arg Ser Gly Lys Ile Met Arg Arg Val Leu Arg Lys Ile Ala Gln
            660             665             670
Asn Asp His Asp Leu Gly Asp Met Ser Thr Val Ala Asp Pro Ser Val
        675             680             685
Ile Ser His Leu Phe Ser His Arg Cys Leu Thr Ile Gln
690             695             700
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<210> 27
<211> 1255
<212> DNA
<213> Homo Sapien
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gtttgtcctc cgggttctgg gatcctgctg gaagagggga gcttctgcaa tgggagttgc 180
cacaaccctg cagcccccaa ccacttccaa aaccttgcag aagcagcatc tagaagcagt 240
gggcgcctac caatatgtgc tcactttcct cttcatgggc cctttcttct cccttcttgt 300
ctttgtcctc ctcttcacgt cactctggcc cttctctgtt ttttacttgg tgtggctcta 360
tgtggactgg gacacaccca accaaggtgg aaggcgttcg gagtggataa ggaaccgggc 420
aatttggaga caactaaggg attattatcc tgtcaagctg gtgaaaacag cagagctgcc 480
cccggatcgg aactacgtgc tgggcgccca ccctcatggg atcatgtgta caggcttcct 540
ctgtaatttc tccaccgaga gcaatggctt ctcccagctc ttcccggggc tccggccctg 600
gttagccgtg ctggctggcc tcttctacct cccggtctat cgcgactaca tcatgtcctt 660
tggactctgt ccggtgagcc gccagagcct ggacttcatc ctgtcccagc cccagctcgg 720
gcaggccgtg gtcatcatgg tggggggtgc gcacgaggcc ctgtattcag tccccgggga 780
gcactgcctt acgctccaga agcgcaaagg cttcgtgcgc ctggcgctga ggcacggggc 840
gtccctggtg cccgtgtact cctttgggga gaatgacatc tttagactta aggcttttgc 900
cacaggctcc tggcagcatt ggtgccagct caccttcaag aagctcatgg gcttctctcc 960
ttgcatcttc tggggtcgcg gtctcttctc agccacctcc tggggcctgc tgcccttttgc 1020
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ggagcacaag gaaagctgtg gggtccccgc ttccacctgc ctcaccttca tctaggcctg 1200
gccgcggcct ttcgctgagc ccctgagccc aaggcactga gacctcaccc actgt 1255
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<210> 28
<211> 341
<212> PRT
<213> Homo Sapien
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<400> 28
Met Gly Val Ala Thr Thr Leu Gln Pro Pro Thr Thr Ser Lys Thr Leu
1               5               10              15
Gln Lys Gln His Leu Glu Ala Val Gly Ala Tyr Gln Tyr Val Leu Thr
            20              25              30
Phe Leu Phe Met Gly Pro Phe Phe Ser Leu Leu Val Phe Val Leu Leu
```

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                35                    40                    45
Phe Thr Ser Leu Trp Pro Phe Ser Val Phe Tyr Leu Val Trp Leu Tyr
    50                    55                    60
Val Asp Trp Asp Thr Pro Asn Gln Gly Gly Arg Arg Ser Glu Trp Ile
65                    70                    75                    80
Arg Asn Arg Ala Ile Trp Arg Gln Leu Arg Asp Tyr Tyr Pro Val Lys
                85                    90                    95
Leu Val Lys Thr Ala Glu Leu Pro Pro Asp Arg Asn Tyr Val Leu Gly
            100                   105                   110
Ala His Pro His Gly Ile Met Cys Thr Gly Phe Leu Cys Asn Phe Ser
        115                   120                   125
Thr Glu Ser Asn Gly Phe Ser Gln Leu Phe Pro Gly Leu Arg Pro Trp
    130                   135                   140
Leu Ala Val Leu Ala Gly Leu Phe Tyr Leu Pro Val Tyr Arg Asp Tyr
145                   150                   155                   160
Ile Met Ser Phe Gly Leu Cys Pro Val Ser Arg Gln Ser Leu Asp Phe
                165                   170                   175
Ile Leu Ser Gln Pro Gln Leu Gly Gln Ala Val Val Ile Met Val Gly
            180                   185                   190
Gly Ala His Glu Ala Leu Tyr Ser Val Pro Gly Glu His Cys Leu Thr
        195                   200                   205
Leu Gln Lys Arg Lys Gly Phe Val Arg Leu Ala Leu Arg His Gly Ala
    210                   215                   220
Ser Leu Val Pro Val Tyr Ser Phe Gly Glu Asn Asp Ile Phe Arg Leu
225                   230                   235                   240
Lys Ala Phe Ala Thr Gly Ser Trp Gln His Trp Cys Gln Leu Thr Phe
            245                   250                   255
Lys Lys Leu Met Gly Phe Ser Pro Cys Ile Phe Trp Gly Arg Gly Leu
        260                   265                   270
Phe Ser Ala Thr Ser Trp Gly Leu Leu Pro Phe Ala Val Pro Ile Thr
    275                   280                   285
Thr Val Val Gly Arg Pro Ile Ala Val Pro Gln Arg Leu His Pro Thr
    290                   295                   300
Glu Glu Glu Val Asn His Tyr His Ala Leu Tyr Met Thr Asp Leu Glu
305                   310                   315                   320
Gln Leu Phe Glu Glu His Lys Glu Ser Cys Gly Val Pro Ala Ser Thr
            325                   330                   335
Cys Leu Thr Phe Ile
        340
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<210> 29
<211> 1365
<212> DNA
<213> Homo Sapien

<400> 29

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tatggaactg ggcgggtaga tggaatcctg agcgaggaca agctgactat tggtggaatc 480
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gcccattttg atgggatatt gggcctcggt tttcccattc tgtctgtgga aggagttcgg 600
cccccgatgg atgtactggt ggagcagggg ctattggata agcctgtctt ctccttttac 660
ctcaacaggg accctgaaga gcctgatgga ggagagctgg tcctgggggg ctcggacccg 720
gcacactaca tcccacccct caccttcgtg ccagtcacgg tccctgccta ctggcagatc 780
cacatggagc gtgtgaaggt gggcccaggg ctgactctct gtgccaaggg ctgtgctgcc 840
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<210> 30
<211> 420
<212> PRT
<213> Homo Sapien

<400> 30
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Leu Asn Val Glu Pro Ser Gly Ala Thr Leu Ile Arg Ile Pro Leu His
             20   25   30
Arg Val Gln Pro Gly Arg Arg Ile Leu Asn Leu Leu Arg Gly Trp Arg
         35   40   45
Glu Pro Ala Glu Leu Pro Lys Leu Gly Ala Pro Ser Pro Gly Asp Lys
     50   55   60
Pro Ile Phe Val Pro Leu Ser Asn Tyr Arg Asp Val Gln Tyr Phe Gly
 65   70   75   80
Glu Ile Gly Leu Gly Thr Pro Pro Gln Asn Phe Thr Val Ala Phe Asp
                 85   90   95
Thr Gly Ser Ser Asn Leu Trp Val Pro Ser Arg Arg Cys His Phe Phe
                100   105   110
Ser Val Pro Cys Trp Leu His His Arg Phe Asp Pro Lys Ala Ser Ser
            115   120   125
Ser Phe Gln Ala Asn Gly Thr Lys Phe Ala Ile Gln Tyr Gly Thr Gly
        130   135   140
Arg Val Asp Gly Ile Leu Ser Glu Asp Lys Leu Thr Ile Gly Gly Ile
145   150   155   160
Lys Gly Ala Ser Val Ile Phe Gly Glu Ala Leu Trp Glu Pro Ser Leu
                165   170   175
Val Phe Ala Phe Ala His Phe Asp Gly Ile Leu Gly Leu Gly Phe Pro
            180   185   190
Ile Leu Ser Val Glu Gly Val Arg Pro Pro Met Asp Val Leu Val Glu
        195   200   205
Gln Gly Leu Leu Asp Lys Pro Val Phe Ser Phe Tyr Leu Asn Arg Asp
    210   215   220
Pro Glu Glu Pro Asp Gly Gly Glu Leu Val Leu Gly Gly Ser Asp Pro
225   230   235   240
Ala His Tyr Ile Pro Pro Leu Thr Phe Val Pro Val Thr Val Pro Ala
                245   250   255
Tyr Trp Gln Ile His Met Glu Arg Val Lys Val Gly Pro Gly Leu Thr
            260   265   270
Leu Cys Ala Lys Gly Cys Ala Ala Ile Leu Asp Thr Gly Thr Ser Leu
        275   280   285
Ile Thr Gly Pro Thr Glu Glu Ile Arg Ala Leu His Ala Ala Ile Gly
    290   295   300
Gly Ile Pro Leu Leu Ala Gly Glu Tyr Ile Ile Leu Cys Ser Glu Ile
305   310   315   320
Pro Lys Leu Pro Ala Val Ser Phe Leu Leu Gly Gly Val Trp Phe Asn
                325   330   335
Leu Thr Ala His Asp Tyr Val Ile Gln Thr Thr Arg Asn Gly Val Arg
            340   345   350
Leu Cys Leu Ser Gly Phe Gln Ala Leu Asp Val Pro Pro Pro Ala Gly

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                355                        360                    365
Pro Phe Trp Ile Leu Gly Asp Val Phe Leu Gly Thr Tyr Val Ala Val
    370                        375                    380
Phe Asp Arg Gly Asp Met Lys Ser Ser Ala Arg Val Gly Leu Ala Arg
385                        390                    395                    400
Ala Arg Thr Arg Gly Ala Asp Leu Gly Trp Gly Glu Thr Ala Gln Ala
                405                        410                    415
Gln Phe Pro Gly
        420
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(54) Title: METHODS AND COMPOSITIONS TO TREAT CARDIOVASCULAR DISEASE USING 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 OR 93804

(57) Abstract: The present invention relates to methods for the diagnosis and treatment of cardiovascular disease, including, but not limited to, atherosclerosis, reperfusion injury, hypertension, restenosis, arterial inflammation, thrombosis and endothelial cell disorders. Specifically, the present invention identifies the differential expression of 139, 258, 1261, 1486, 2398, 2414, 7660, 8587, 10183, 10550, 12680, 17921, 32248, 60489 or 93804 genes in cardiovascular disease states, relative to their expression in normal, or non-cardiovascular disease states, and/or in response to manipulations relevant to cardiovascular disease. The present invention de-scribes methods for the diagnostic evaluation and prognosis of various cardiovascular diseases, and for the identification of subjects exhibiting a predisposition to such conditions. The invention also provides methods for identifying a compound capable of modu-lating cardiovascular disease. The present invention also provides methods for the identification and therapeutic use of compounds as treatments of cardiovascular disease.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/35538

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(7)   :   A61K 49/00; A01N 25/00; C12Q 1/68
US CL   :   424/9.2; 514/824; 435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/9.2; 514/824; 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PYNE et al. Sphingosine 1-phosphate signalling via the endothelial differentiation gene family of G-protein-coupled receptors. Pharmacology & Therapeutics. November 2000, Vol. 88, No. 2, pages 115-131, see entire document.	1-14
X --- Y	HLA et al. An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to G-protein-coupled receptors. Journal of Biological Chemistry. 05 June 1990, Vol. 265, No. 16, pages 9308-9313, see entire document.	1-8 ---------- 9-14
X --- Y	MATSUNO et al. Induction of lasting complete regression of preformed distinct solid tumors by targeting the tumor vaculature using two new anti-endoglin monoclonal antibodies. Clinical Cancer Research. February 1999, Vol. 5, No. 2, pages 371-382, see entire document.	9-14 ------- 1-18
Y	GenBank Database on NCBI, US National Library of Medicine, (Bethesda, MD, USA), Accession Number M31210, HLA et al. 'An abundant transcript induced in differentiating human endothelial cells encodes a polypeptide with structural similarities to G-protein-coupled receptors' 07 November 1994, enitre nucleic acid sequence.	1-14

☐ Further documents are listed in the continuation of Box C.   ☐   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 March 2004 (31.03.2004)	13 APR 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Monika Sheinberg Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
Electronic Journals, WEST
search terms: gi#181948, gi#181949, edg-1, edg, therapy, treatment, vascular, atherosclerosis, modulation, induction

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